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Some Factors Affecting the Flesh Quality of Salmonids: Pigmentation, Composition and Eating Quality

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A thesis submitted to the University of Bristol in accordance with the requirements of the degree of DOCTOR OF PHILOSOPHY in the Faculty of Medical Sciences

March, 1998

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Abstract

This thesis is concerned with identifying some of the factors which affect the flesh quality of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). The flesh of any food animal is of utmost importance. Much work has been carried out on the flesh of land animals, but comparatively little is known about the factors which affect the quality of fish flesh. Salmonids have been farmed for a relatively short period of time, but great advances have been made in the techniques for their rearing. Now that the fish can be grown easily, more information is required on the factors which affect their flesh quality in order to produce a consistent product.

Fish fed a high oil diet have a significantly higher level of flesh lipid than fish of the same size fed a low oil diet (uncertainty $p<0.001$). This has implications for the eating quality of the fish as many attributes of the eating quality of smoked salmon are significantly affected by the flesh lipid content. The flesh texture becomes softer and the flavours stronger, apart from *salty* flavour which decreases dramatically with increasing lipid ($p<0.001$). The general effect of increasing lipid is to increase the perception of overall flavour and the overall liking of the product, as judged by the ten person trained taste panel. In contrast there are much fewer effects of the lipid content on cooked salmon and there are no effects on the overall flavour or overall liking ($p>0.05$).

The colour of the flesh is of great importance to the flesh quality of salmonids. Stress at slaughter is known to affect the muscle chemistry post-slaughter. This work shows that reducing stress at slaughter significantly increases the colour of the flesh, resulting in lower lightness ($p<0.05$), an increased red hue ($p<0.05$) and reduced opacity ($p<0.05$) as measured using the CIElab 1976 method. The change in colour is also shown by an increased Roche colour card score using the subjective colour card score ($p<0.05$). Reduced stress at slaughter is also found to result in a longer time to the onset of rigor ($p<0.001$) and a reduced susceptibility to gaping of the flesh ($p<0.01$). Current commercial 'best practice' methods of slaughter were found to be highly stressful to the fish.

The red colour of the flesh has been previously reported to reduce during storage, but this research found no changes in the level of the pigment astaxanthin during 12 days of storage of salmon fillets on ice. No effects of the level of astaxanthin or the anti-oxidant vitamins ascorbic acid and α-tocopherol at slaughter were found on the eating quality of the cooked flesh, either fresh or after 12 days storage on ice. However, many other attributes of the eating quality were significantly affected during the storage period, with increases in many flavours and textures associated with "off" fish. This translated into a significant reduction in the expression of overall liking by the trained taste panel ($p<0.001$).

The research for this thesis has shown areas where further research is required to investigate factors having effects on flesh quality. The most important of these new areas is the effect of stress level at slaughter on flesh quality. There is a strong possibility that stress at this point has wide ranging effects, the results of which may actually confound improvements introduced into in other areas of quality control unless they are eliminated from salmon farming practices.

Acknowledgements

A large number of people have helped me through this project and I would like to thank them all. First I must thank my sponsors, F. Hoffmann- La Roche, who kindly funded the entire project. I would especially like to thank Dr Ludwig Völker who initiated the project and his successor Dr Jacques Gabaudan. Roche U.K. were also actively involved with the project. Dr Paul Beardsworth kindly provided the vitamin packages for the diets in chapters 3 and 5 and a lot of important advice on their formulations. His successor Dr John Springate was also very supportive of my work. I must also thank my two supervisors at the University of Bristol, Steve Kestin and Dr Paul Warriss, for their guidance and support, despite the smell of fish!

During the course of the project Hugh and Mary Horrex of Landlocked Salmon (Europe) were very kind and supportive and introduced me to many members of the salmon and trout industry with whom I was to later work. They also supplied the facilities for the experiments on rainbow trout outlined in chapters 2 and 4.

For the experiments on Atlantic salmon, Marine Harvest McConnell Ltd. rented their trials site at Loch Eil to us and kindly supplied the fish for the salmon slaughter trial in chapter 4. However, none of the salmon projects there would have been possible without the guidance of the technical manager, David Mitchell, and the on-site supervision of Billy Elder. The post-slaughter handling of the fish through the factory was facilitated by Ian Michie, despite all of the troubles associated with an experiment taking place next to an industrial line.

Feed companies were involved in all of the experiments. The experiments in chapter 2 used diets made at the EWOS Technology Centre by Niall MacDonald. The trial itself was part sponsored by Dr René Blum of Lonza. The diets for chapter 3 and

chapter 5 were made by BOCM Pauls Ltd. under the supervision of Richard McKinney. I am also grateful to Richard for his advice on the formulation of the diets and on other matters. Finally, Trouw (UK) Ltd. kindly funded the experiment on the slaughter of salmon in chapter 4 and Rob Sinnott and Deirdre Lee provided a lot of advice and assistance during the course of that experiment.

The whole of the staff in the Division of Food Animal Science in the University of Bristol have put up with the smell of fish for three years. However, some members of staff have been more closely involved. Geoff Nute, Ann Baker and Sue Hughes supervised the taste panelling of the fish in chapters 3 and 5 and Geoff and Sue assisted me with the data analysis. Dr Toby Knowles advised me on the statistical analyses required for some of the work. Technical assistance in the field was given by Andrew Phillips, Simon Adams, Simon Williams, Steve Brown and Lindsay Wilkins. Rose Ball and Paula Gibbs were of great help with the chemical analyses — especially with the work required for the preparation and running of the HPLC.

Having completed the experimentation, the thesis would have never taken the form it has without a great deal of comments and proof reading — not always favourable — from Dr Claire Weeks, Dr Paul Warriss, Steve Kestin and my parents. This was perhaps the hardest part of the whole work — certainly the least enjoyable!

Declaration

The work in this thesis is that of the author alone, except where acknowledged. The material has not been previously presented for any other higher degree to the University of Bristol or elsewhere.

All experimental work was carried out to comply with the Animals (Scientific Procedures) Act 1986.

Signed  David H.F. Robb

Dated 7th August 1998

Contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures

Chapter 1

Chapter 2

Introduction

This study of the flesh quality of farmed Atlantic salmon (*Salmo salar*) is an examination of the factors which give the flesh of the fish its characteristic appearance, taste and texture. In salmonids the characteristics of flesh quality as perceived by the commercial market are determined by the appearance, composition, eating quality and texture of the flesh. Although the growth and husbandry of salmonids has been extensively studied, comparatively little research has been published on their flesh quality.

Factors affecting the flesh quality require investigation in order to allow the salmon farming industry to improve its product. Information from such studies will also enable variations in quality to be reduced, minimising the number of fish downgraded because they are outside the product specifications.

Because of the small number of detailed studies in this area, the thesis has become, of necessity, a broad ranging work investigating the effects of diets, pre-slaughter handling and the flesh composition at slaughter on the flesh quality. The quality of the fish is measured by the chemical composition of the flesh, by its visual appearance and by its eating quality.

The investigation of the chemical composition of the flesh concentrates on the proximate composition — the levels of dry matter, ash, protein and lipid in the flesh. The levels of three anti-oxidants are also of especial interest. Two of these, vitamin C and vitamin E, are of great biological importance. The third is the carotenoid pigment astaxanthin, which gives the characteristic 'red' colour to the flesh and may also have biological significance. The colour is of great importance to the appearance of the flesh and is commonly measured using a subjective scoring system or by a colour

meter. Finally, knowledge of factors affecting the flavour and texture of the fish is essential. The eating quality of the flesh is assessed by sensory profiling, which uses descriptive terms to build up information on the textures and flavours associated with the samples. This profile can be used in order to determine the nature of the changes caused by the different treatments in experimentation.

The first chapter of the thesis summarises the life-cycle of the Atlantic salmon and how it has been adapted to aquaculture. This provides an explanation of contemporary methods of salmon farming and indicates those points in the life-cycle when the flesh quality may be affected. The types of diets fed to farmed fish are also summarised in this chapter, to give background information on their ingredients and manufacture. This will allow the reader to understand terms used later on in the experimental chapters. Finally, the first chapter reviews the scientific literature on the factors affecting flesh quality, both in Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*), which have very similar flesh characteristics. Wherever relevant, work on other food animal species is reviewed in order to consider ideas which may also be applicable to salmonids.

Chapters 2 to 5 cover experimental research into some of the factors affecting quality highlighted in the literature review in chapter 1. Chapter 2 examines the effects of raising the level of oil in the diet on the level of lipid and on other parameters in the flesh. This is a very topical issue as the salmon industry looks set to increase the level of fish oil used in the diets.

The level of lipid is shown to be altered by the level of oil in the diet and it is very important to understand how this may affect the consumers' perception of the product. The effects of changes in lipid levels in the flesh on the eating quality of the salmon are therefore investigated in chapter 3. Eating quality assessments were carried out

both on cooked flesh and on smoked flesh — a product of great economic value to the salmon farming industry.

Extensive research on red meat species has shown that pre-slaughter handling has a major effect on the quality of the flesh of these animals. Slaughter potentially is a particularly stressful time for fish and many of the quality parameters affected by pre-slaughter handling and slaughter techniques in red meat species — such as appearance and texture — are also important for salmon flesh quality. Chapter 4 therefore examines pre-slaughter handling and the slaughter techniques used for salmon and their effects on the flesh quality.

Chapter 5 investigates changes in flesh quality during storage of the fresh flesh on ice. This is a common method for storing fish prior to processing or sale. The aim is to investigate the effects of three anti-oxidants in the flesh on the eating quality of the fish and on the level of the carotenoid pigment astaxanthin which gives the salmon their characteristic red colour.

The findings of the experiments and their implications are discussed in chapter 6. This provides the industry with further information on various factors which have an important effect on flesh quality. It also highlights where further research is required to follow up on the conclusions drawn from this thesis.

Chapter 1

Background and Literature Review

1.1 Fish Farming

Farming fish for food was known in ancient China, where wild caught carp (*Cyprinus carpio*) were kept in ponds until they were required, resulting in a ready supply of fresh fish all year round. The methods used were crude, but were slowly refined to enable the fish to breed in captivity.

Fish farming in Europe began with the farming of carp by the Romans and developed in the Middle Ages when monasteries maintained ponds stocked with fish such as carp (Balon, 1995). This resulted in a steady supply of fish for the monks, even in winter, when wild fish were extremely hard, if not impossible, to catch. However, the relatively small urban populations could be supported by land-based agriculture and by wild fish caught from the sea. Thus, fish farming remained largely unaltered until the late nineteenth century when trout farming began.

Trout farming started in ponds, initiating the development of many of the techniques used in modern salmon and trout farming. The most important of these was the husbandry required to artificially spawn, fertilise and incubate the eggs — a process which has changed little over the years. In the 1950's the large-scale intensive culture of salmon, mainly the Atlantic salmon, was embarked on. Early attempts were not very successful and the growth of the fish was slow as they were mainly fed on a diet of offal from meat processing plants. Over the next few decades improvements in diets and farming techniques led to the rapid growth of the salmon farming industry, firstly in Norway, then in Scotland, Western Europe, Canada and the United States, before spreading to the southern hemisphere in Australia, New Zealand and Chile (currently the second largest producer of Atlantic salmon after Norway).

Modern salmon farming is an important industry, supplying a large amount of a high quality food product. Salmon flesh is versatile, being used for "value added" products

such as smoked salmon, gravadlax and patés. The range of products greatly increases the potential market for the fish, but also results in strict quality constraints being imposed with the appearance of the product assuming increasing importance.

The increase in supply of farmed salmon has lead to a decrease in the price of the fish, making it available to more consumers. The majority of the fish produced in the United Kingdom is destined to be smoked, for the higher value end of the market. However, the perceived intrinsic healthiness of the fresh fish also results in large amounts of the unprocessed fresh fish being bought by the consumer.

1.2 Life-cycle of Wild Salmon

1.2.1 Adult Salmon

Atlantic salmon are anadromous — their life-cycle is split into two distinct phases.

Adult salmon feed in the sea, where they make the most rapid growth, making use of the plentiful supply of food. The fish are voracious feeders near the top of the food chain, with crustaceans and smaller fish forming their diet. After one or two years of growth at sea they start to mature sexually. As their gonads develop, the fish migrate back towards the river where they were spawned. The fish are thought to home in on the correct river using the "smell" of the water. During the migration the gonads develop further and the fish develop secondary sexual characteristics. Both sexes, but especially the male, turn from the silver colour they have at sea to brown as they enter freshwater between spring and autumn. The males also develop a kype, a dramatically elongated lower jaw, which is used for display to females and for driving off rival males from the spawning beds or redds.

1.2.2 Spawning

The adult salmon are motivated by a powerful drive to swim up the river, even jumping up high waterfalls to get to the gravel beds where they will spawn. On these redds the males compete for access to mate with females. During her several spawnings the female carves out a groove in the gravel with her body. The male then comes alongside her to fertilise the eggs as she spawns them. The female covers the fertilised eggs with gravel and searches for another place to spawn. This allows her to mate with several males thus spreading the risk to her eggs, which are very susceptible to disturbance during early development.

After spawning, the spent adults may die from exhaustion, having used most of their body reserves during the migration and for developing their gonads. However, unlike some North American species of salmon, the Atlantic salmon can recover and will often return to the sea, where they feed until the next year when they return to spawn again. During this recovery period they are referred to as kelts.

1.2.3 Eggs

Protected by the covering of gravel, the eggs slowly develop. After a period of time, generally four to eight weeks depending on the water temperature, the form of the embryo, especially the eyes, can be seen through the wall of the egg, and the egg is said to have eyed. The wall of the egg at this time is quite thick and the developing embryo is much less susceptible to damage from movement than before eyeing. When the embryo hatches out, three to six weeks after eyeing, a free swimming larva, or alevin, emerges beneath the gravel. The time from spawning to hatching is temperature dependent and is generally about 350 degree days (a product of temperature and time, for instance 24 hours at 10°C is 10 degree days).

1.2.4 Alevins, Fry and Parr

The alevin has a large yolk sac filled with protein and oil from which it receives its nutrients. Once the yolk has been absorbed the larva is able to feed and is referred to as a fry. The fry start to emerge from the protection of the gravel in late spring, feeding on zooplankton and small crustaceans and insects. Their skins darken to a brown colour and they are recognisable as fish. As they grow further, small bluish vertical bars appear on their flanks. These are called parr marks and the fish are referred to as parr. The parr over-winter in the river and continue to grow the next spring. By late summer in the first year, two distinct groups of parr can be observed, one group consisting of much larger individuals. These fish, known as smolts, swim

to the sea to join the adult population in April of their second year. The population of smaller fish stay another winter in freshwater, before going to sea the following spring, when they will be large enough to survive the journey and complete the life-cycle.

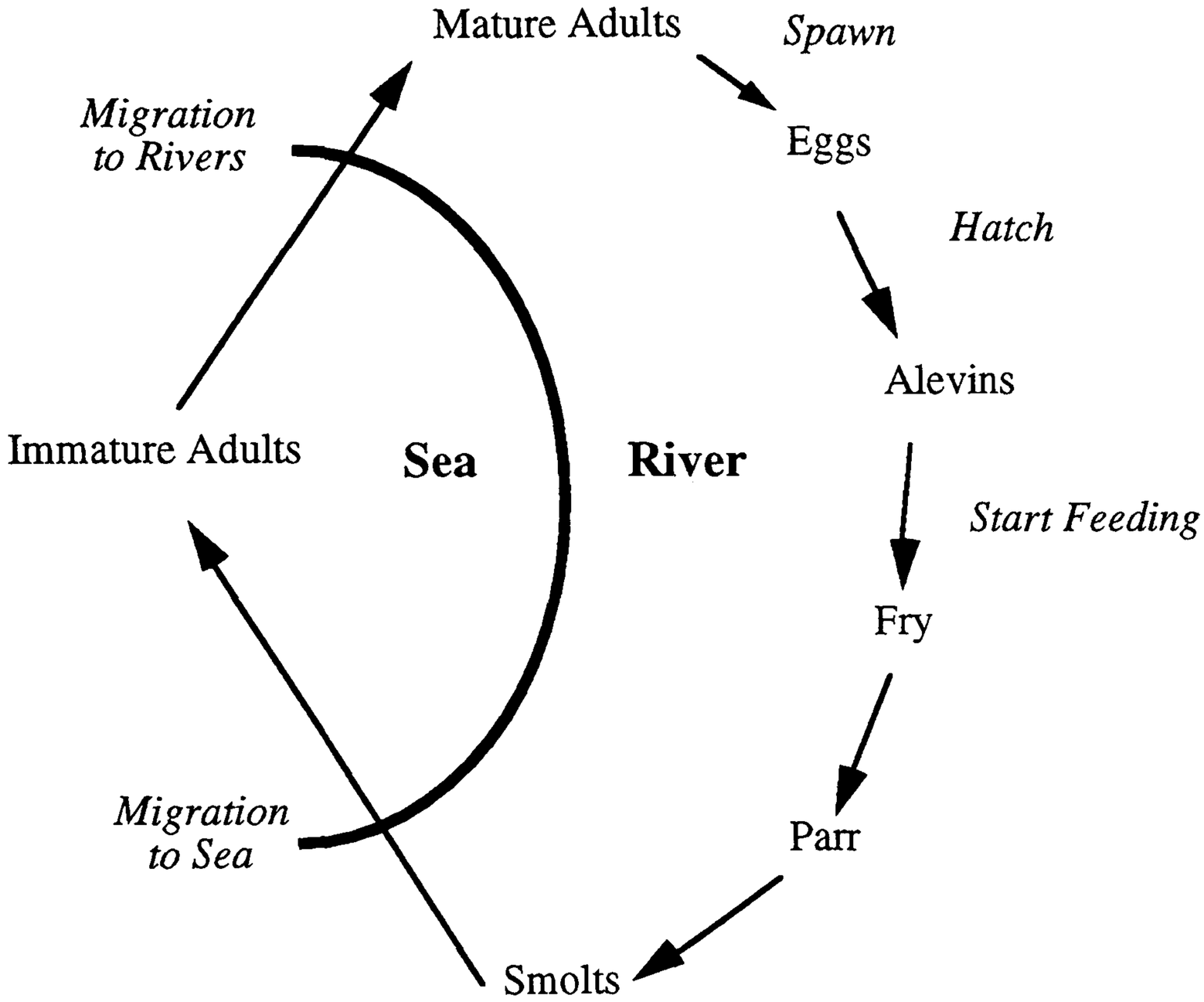

Figure 1.1 Summary of the life-cycle of Atlantic salmon in the wild.

1.3 Farming Atlantic Salmon

The farming of Atlantic salmon is governed by the life-cycle of the wild fish. The broodstock are kept in cages in the sea and receive specially formulated diets to improve the quality of their eggs. As spawning time approaches, generally in the late autumn, they are usually moved to freshwater tanks to finish maturing.

1.3.1 Eggs

The eggs are stripped by hand into buckets by squeezing the body cavity so as to expel the eggs through the vent. The sperms are added and the two mixed with water by hand to activate the sperms and ensure an even fertilisation. After ten minutes, the excess sperms are washed off and the eggs transferred to the incubation trays. The eggs are incubated in tanks in the dark on wire or plastic mesh screens, or in large plastic hoppers which allow water to circulate freely around the eggs, supplying oxygen and washing away waste products. The trays are checked 24 hours after fertilisation to remove unfertilised or dead eggs, which become an opaque white. They then remain unhandled until eyeing, but during this time they may be treated with chemotherapeutics such as formalin and Chloramin T to control the build-up of bacteria and fungi.

After eyeing, the eggs can be safely handled again and they are "picked" to remove dead ones. They are also gently rolled between two surfaces to free the shells from their covering of bacteria and fungi and to "shock" the eggs causing any weak eggs to die at this point, rather than later. The eggs are returned to their trays and picked regularly until hatching. On hatching, the alevins drop through the screens to the base of the tanks, where they are provided with some cover under which to hide whilst they absorb their yolk sacs. After the absorption of the yolk sacs, the fry start to feed and the cover is removed allowing them to swim freely about the tank.

1.3.2 Fry / Parr

The fry are kept in freshwater tanks or raceways once they start to feed and are regularly graded to separate the large from the small ones. This minimises the effects of feeding hierarchies, where the biggest fish will prevent the smaller ones from obtaining sufficient food for maximum growth. The fish are fed manufactured diets, which encourage rapid growth and hence early smolting. Some of the parr are also exposed to photoperiodic control. This causes smolting to occur at different times of the year, resulting in the potential to supply fish to sea almost throughout the year.

1.3.3 Smolting and Growth at Sea

On smolting, the fish are transferred to seawater cages, either by lorry and boat or by helicopter. Once in the sea, the fish experience an increase in growth rate and rapidly put on weight, reaching market size (3-5kg) within about eighteen months of transfer. During this period the fish are regularly treated against disease and parasites, which would otherwise thrive uncontrolled owing to the high density of the fish. These include furunculosis (*Aeromonas salmonicida*), infectious pancreatic necrosis (a viral disease) and sea lice (*Lepeophtheirus* sp. and *Caligus* sp.). Recent developments in husbandry have helped to minimise the effects of disease, but parasites are still a problem and many treatments are still required.

1.3.4 Diets

During the development of the salmon farming industry, the manufacturing of diets for the fish has changed greatly. Early diets were commonly made from waste offal from meat processing plants. Later, wet diets were made by mixing fish oil with protein (largely from fish meal) and carbohydrates. Vitamin packages could also be added to the mix to meet the nutritional requirements of the fish. The diets were

passed through a mill to cut them into pellets which could be easily fed to the fish. Such diets were able to hold up to about 16% oil, enabling faster growth of the fish than before.

Recent advances in feed technology have allowed the level of oil in the diets to be radically increased. Starch is added to the fish meal and oil mix. The mixture is then cooked with steam, causing the starch to crack. This forms new sites for the oil to be absorbed by the carbohydrate, allowing the levels of oil in the finished diet to reach over 30%. If further oil is sprayed on the mix after extrusion, the levels of oil achieved are as high as 36% and it is predicted that 40% oil diets will soon be commercially attainable by adding oil under vacuum.

The nutrient balance of the diets is carefully monitored by the manufacturing companies to achieve the maximum growth and health of the fish. Protein complements are checked and vitamin packages added to the diets. Either of the carotenoid pigments astaxanthin or canthaxanthin are included in the vitamin package. These provide the red colouration of the flesh that is characteristic of salmon.

Diets are obviously of prime importance in promoting flesh quality, as they affect the composition of the fish. The constituents of the diet can be split up into three main groups, not including carbohydrates which have little nutritional significance to salmonids (Driedzic and Hochachka 1978). These are oil, protein and the micro-nutrients:

i) Dietary Oil

The dietary oil in the feed for salmonids is derived mainly from fish oil. The oil is extracted from fish very soon after they are caught. The freshly caught fish are cooked and then pressed, releasing the liquids — the remainder is the fish meal. The liquids are centrifuged twice, resulting in the extraction of the fish oil, with the solid and aqueous phases removed (Barlow, 1997). The oil is stored until required for the production of the fish diets. It is then remixed with fish meal and other ingredients to make the required formulation. Extra oil can also be sprayed on to expanded diets to increase the inclusion still further.

Fish oil is high in (n-3) polyunsaturated fatty acids (PUFAs), especially the long chain (n-3) PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Sargent, 1996). These PUFAs have been implicated in the reduction of the occurrence of cardiovascular disease in humans and are considered essential to the human diet. Fish oil is the only practical source of these PUFAs and the high level of fish oil in the manufactured salmonid diets ensures that the farmed fish have a high content. This results in a product which has a high value to human nutrition. High dietary levels of these PUFAs also results in rapid growth of the fish compared to diets with higher levels of shorter chain (n-3) PUFAs and (n-6) PUFAs. The use of alternative dietary oils, such as soya oil, is being investigated for salmonids (Roberts and Talbot, 1997) but many of these do not have such high levels of the (n-3) PUFAs and so the final products may not have such a high nutritional value.

Fish can use both dietary lipid and protein as energy sources (Driedzic and Hochachka, 1976). When dietary oil levels are low, dietary proteins are deaminated and the amino acids are used for energy. As the economic cost of dietary protein is higher than that of dietary oil, it is advantageous to have the oil level such that the diets do not become oil deficient at any stage. Higher oil levels also result in faster

growth rates. This has an economic advantage, in that the fish take less time to reach harvest weight and therefore cost less to raise in terms of overheads.

ii) Dietary Protein

The protein in manufactured diets for salmonids comes mostly from fish meal, although current research is investigating the possibility of using alternative protein meals such as soya meal (Roberts and Talbot, 1997). The fish meal is generated when the oils are removed in the process described above, resulting in a high quality meal known as LT fish meal (LT stands for low temperature). This protein meal is high in the essential amino acids required by fish and therefore it is converted easily into flesh, resulting in good growth. The most commonly used meal is LT94 which has a 94% digestibility rating for fish. The meal is used to produce diets with protein contents of 47% to 54% for the fresh water stage of the life cycle and 39% to 46% for the sea water stage (Anon., 1997).

Debate about the future of fish diets frequently refers to production of a so-called "square diet". This means a diet with a protein content of 40% and an oil content of 40%. It is hoped that this will be the most economic way of producing a fast growing fish, which will quickly reach market size.

iii) Micro-Nutrients

The addition of micro-nutrients to the diets is essential for the growth and health of farmed fish. A great deal of research has already been carried out into the levels of vitamins and minerals required for the fish and this is taken into consideration by the feed companies when formulating diets. These requirements were reviewed in the publication by Steffens (1989) and by the USA's National Research Council (1993).

Deficiency in some of the micro-nutrients, such as the vitamins, will initially cause reduced growth, and chronic deficiency may eventually result in mortality. Other micro-nutrients in the diet include carotenoid pigments which have biological significances for both eggs and fry (Robb *et al.*, 1995 and Christiansen, 1996 respectively). In larger fish the pigments are deposited in the muscle, causing the red colouration of the flesh. This colouration is required by the industry as the consumer expects the flesh to be 'salmon pink'. The pigments are generally chemically synthesised, although natural products containing astaxanthin are also available. The synthesised products are protected by a starch coating which minimises the chance of their oxidation during the processing and storage of the feed.

1.3.5 Maturity

At the beginning of the second summer at sea, some of the fish start to mature sexually. Termed grilse, these fish show a growth burst as they accumulate energy stores and start to develop the external signs of maturity described in section 1.2.1. Later, as the fish mature further, they stop feeding and their gonads develop rapidly. Nutrients stored in the flesh are channelled into the maturing gonads, causing the properties of the flesh to change. The pigment and lipids in particular are mobilised from the flesh and this results in the moisture level of the flesh increasing and the red colour reducing. The flesh also becomes softer and is generally of very poor quality. It is therefore important that the grilse are killed before they reach this stage. In Britain grilse are graded out and killed by the end of August, but even as early as July the quality begins to decrease (pers. comm., D. Mitchell, Marine Harvest McConnell Ltd.).

1.3.6 Slaughter

Photoperiod manipulation of smolts helps to provide a year round supply of fish ready for market, but the supply peaks in the autumn. On reaching the required market weight, the fish are slaughtered, either by a blow to the head (percussion stunning) or by anaesthesia using carbon dioxide. Both processes are followed by exsanguination which is performed by slitting the gill arches and allowing the blood to flow out. The fish are then placed in a bin of ice slurry to cool, minimising the activity of enzymes in their flesh and viscera, which would otherwise initiate the autolysis of the fish at increased temperatures. In order to further minimise the activity of gut enzymes, the fish are starved for a period of at least seven days before slaughter, which empties the gut of food. This also reduces the risk of damaging the gut during evisceration, which could cause contamination of the carcass.

1.3.7 Processing and Storage

After slaughter, the fish are processed. The type of processing carried out depends on the product required. The fresh fish are eviscerated before the onset of rigor, removing the viscera including the kidneys and the heart. They are then packed in ice and may be transferred to the market where they can be sold whole, as fillets or as steaks. The quality of the steaks and fillets is mainly judged by the colour of the flesh, with the market demanding an even rich red colour. Fish sold unfilleted are required to have a silvery coloured skin, with no scale loss or other signs of damage.

Fish destined for smoking are also eviscerated, generally before rigor sets in. After evisceration they are packed in ice until rigor resolves, then filleted and placed skin down on flat racks. The fillets are cured for a period of approximately 20 hours, either by immersion in brine or by covering them with a layer of dry salt. At the end of curing the fillets are quickly rinsed in freshwater to remove the excess salt and placed

on clean, flat racks. The racks are placed in the smoking kilns where the fillets are exposed to smoke from smouldering wood chips — with various hard woods being used depending on market demands. The smoking can either be 'cold' (about 25˚C) or 'hot' (35˚C) and the time in the kiln varies depending on the product required. After smoking, the fillets are trimmed, to remove the bones and the layer of flesh directly exposed to the smoke, sliced and vacuum packed for storage. The quality of the product is very dependent on the colour of the flesh, which should be an even, rich red. The nature of the product, which is generally sold as thin slices, means that any uneven colouring is very obvious and results in the downgrading of the product.

The autumnal increase in the supply of farmed fish contrasts with the steady year round market demand. This means that some fish are placed in frozen storage until required, either unfilleted or filleted for smoking. During storage the fish are kept at a low temperature (at least -30˚C) in order to minimise the oxidation of the fats .

1.4 Factors Affecting Flesh Quality

In section 1.3, methods of farming salmon were reviewed briefly. The final flesh quality of the fish can be affected at many different points in the process. This section reviews the factors affecting flesh quality and will introduce the hypotheses to be tested in the experimental chapters of the thesis.

1.4.1 Dietary Effects

The composition of the diets fed to the fish in salt water was discussed in section 1.3.4. The diets have major effects on the quality of the fish, especially in determining their growth rate and flesh composition.

1.4.1.1 Growth Rate

The growth rate of the fish is affected by several dietary factors. The size of the ration fed affects the rate of growth, shown by Staples and Nomura (1976) using rainbow trout. A peak rate of gain of weight is reached at a certain ration — which depends on the weight of the fish and the water temperature — and above this there is a disadvantage in increasing the ration as the food is not eaten.

Growth rate is also affected by the ratio of protein to total energy in the feed. Winfree and Stickney (1981) showed that tilapia fry (*Tilapia aurea*) increase their rate of growth as the protein to energy ratio decrease. This may occur because at low energy levels the fish do not get sufficient energy from the dietary oil for normal metabolism and have to use the protein from the diet as an energy source. Garling and Wilson (1976) also showed that for channel catfish fingerlings (*Ictalurus punctatus*) the absolute level of protein and energy is important as well as the ratio of the two.

1.4.1.2 Proximate Composition of the Fish

For convenience the proximate composition of an animal is commonly measured, rather than describing the complete composition. This provides information on the amount of dry matter, ash, protein (measured as nitrogen), lipid and energy within the entire fish or within specific tissues and organs.

The proximate composition is often quoted in percentage terms, enabling easy comparison between samples. However, care should be taken when interpreting such data. The proximate composition may be affected by various factors including ration, dietary protein to energy ratio and dietary oil, which should all be taken into account when investigating the effect of a variable on the proximate composition of fish:

i) Dietary Ration

The chemical composition of the fish is affected by dietary ration and composition (reviewed by Shearer, 1994). The percentage lipid content of the fish was found to increase with increasing ration for rainbow trout fry, juveniles and adults (Staples and Nomura, 1976). This has implications for feeding trials, as it must be ensured that the level of ration consumed is the same for all diets. This level will be set by the least palatable diet.

ii) Dietary Protein to Energy Ratio

Garling and Wilson (1976) in their work on channel catfish fingerlings showed that for a given protein level, increasing the total energy of the diet resulted in increased flesh lipid and decreased flesh protein. Similarly in tilapia fry, an increase in the ratio of dietary protein to energy results in a decrease in body lipid (Winfree and Stickney, 1981).

iii) Dietary Oil

Shearer (1994), in his review paper on factors affecting the proximate composition of farmed fish, found no evidence for increased body lipid levels with increased dietary oil *per se*. He argued that total dietary energy has a far greater effect on whole fish lipid stores. Certainly, dietary oil plays a part in determining the level of fillet lipid, but this was hard to separate from the effect of dietary energy. Thus, although Takeuchi *et al.* (1978) investigated the effects of isonitrogenous diets with increasing levels of dietary oil and found that lipid levels increase, Shearer argued that this was due to the increase in energy. However, salmonids make little use of carbohydrates in their diet and protein is their other main dietary source of energy. Therefore it is probable that the level of dietary oil is important to the composition of Atlantic salmon.

1.4.2 Life Cycle

The stage of the life cycle also affects the composition of the fish flesh. There is an annual cycle of increasing and decreasing lipid levels in the fish (Shearer *et al.*, 1994). During the summer the fish all increase their lipid reserves, ready for the winter, when food is scarce and lipid levels will drop. Lipid levels also change before major events in the life of the fish: smolting, growth and sexual maturity.

i) Smolting

In the period before smolting the fish increase the level of flesh lipid, but at smoltification the levels decrease as the lipids are mobilised (Shearer *et al.*, 1994). The extra energy is required for the migration to sea and to give the fish energy during the period of adaptation from freshwater to sea water. This is a highly stressful period for the fish and often in aquaculture situations they will not feed for a short period

immediately following transfer to saltwater. This is typically only for one day, but fasting may last longer, so the extra store of lipids built up before transfer enables them to survive the short period of starvation. In the wild the store of lipids supplies the fish with energy during the course of the migration, when food supplies may be variable as the fish adapt to their new environment.

ii) Live Weight

As the fish increase in size, the level of fillet lipid increases. Shearer (1994) observed that for fish fed the same diet, regression analysis of the logarithm of the live weight against the logarithm of the total lipid content resulted in a high correlation coefficient (*r* value). Citing data extracted from a paper by Reinitz (1983) Shearer observed that this effect occurred even when rainbow trout were fed different dietary protein levels at different rations ($r^2 = 0.950$). This shows that live weight is a very important factor in determining the fillet fat level, affecting 95% of the observed variation. Similar observations were made for the ash and protein content of the fish (Shearer, 1994).

iii) Sexual Maturity

Fish start to sexually mature about 10 months before spawning. Initially the levels of flesh fat increase, as the fish prepare for maturity and the spawning migration. As the gonads start to develop, the fish channel resources towards the process of maturing and the levels of flesh lipid consequently decrease — partly for the development of the gonads, but mainly to supply the fish with energy after the cessation of feeding (Hardy *et al.*, 1985). With the decrease of flesh lipid content, an increase in moisture is observed. This results in the soft, poor quality flesh associated with mature salmonids.

1.4.3 Husbandry

During the final months of growth prior to slaughter the fish are subjected to different husbandry practices in an attempt to manipulate the quality of their flesh. Two types of manipulation may be used: exercise or starvation.

i) Exercise

Fish can be exercised prior to slaughter in order to attempt to tone their muscles and to reduce their fat levels, making them more like wild fish in their flesh characteristics. Christiansen *et al.* (1989) showed that first feeding Arctic charr fry (*Salvelinus alpinus*) lost body lipid after being exercised for 189 days. However, Poston *et al.* (1969) found no effect on the proximate composition of exercised rainbow trout after 140 days. Wiseman (1993) observed a similar lack of effect on the lipid content of portion size (325-375g) rainbow trout exercised for 18 days in a trout farm raceway. It would therefore appear that exercise probably does not affect the proximate composition of the fish, providing that the exercise is not too severe and the nutrition is adequate. Consequently, exercise is not widely used in salmon farming.

ii) Starvation

Starvation periods are also used by farmers in attempts to manipulate the level of fillet fat prior to slaughter. Fish may be starved for a period of several weeks and even for up to a month, although now the usual period is seven days (FAWC, 1996). During the period of starvation the fish first use their lipid stores and then their protein for energy (Love, 1970). Weatherley and Gill (1981) and Wiseman (1993) also showed the depletion of lipid in rainbow trout during fasting, resulting in an increase in the moisture content.

1.4.4 Environmental Conditions

The ambient photoperiod affects the composition of the fish by triggering the development of maturity. The water temperature also affects the composition of the flesh:

Water Temperature

As the temperature of the water rises, the metabolic rate of the fish increases. Thus at high temperatures the fish may require more energy than they can acquire from their dietary intake. Sockeye salmon (*Oncorhynchus nerka*) were found to be unable to accumulate lipid at high temperatures and showed less growth for a given ration (Brett *et al.* 1969). The point where water temperature starts to affect the composition is species specific and depends on the natural environment of the individual species.

1.4.5 Appearance

The general appearance of food products is of great importance to their quality. Consumers have a preconceived idea of how the product should appear and deviations from this ideal are often viewed with suspicion. Fillets and steaks expose the muscle structure and any damage to this structure will be obvious. Thus either bruising of the flesh or breakage of the muscle fibres (gaping) results in the down grading of both products.

The colour of the salmon flesh is especially important. Consumers demand that salmon flesh should be 'salmon pink' — a red colour derived from carotenoid pigments such as astaxanthin or canthaxanthin (figure 1.2). Both pigments are fed to farmed fish as supplements to manufactured diets in order to achieve the required colouration.

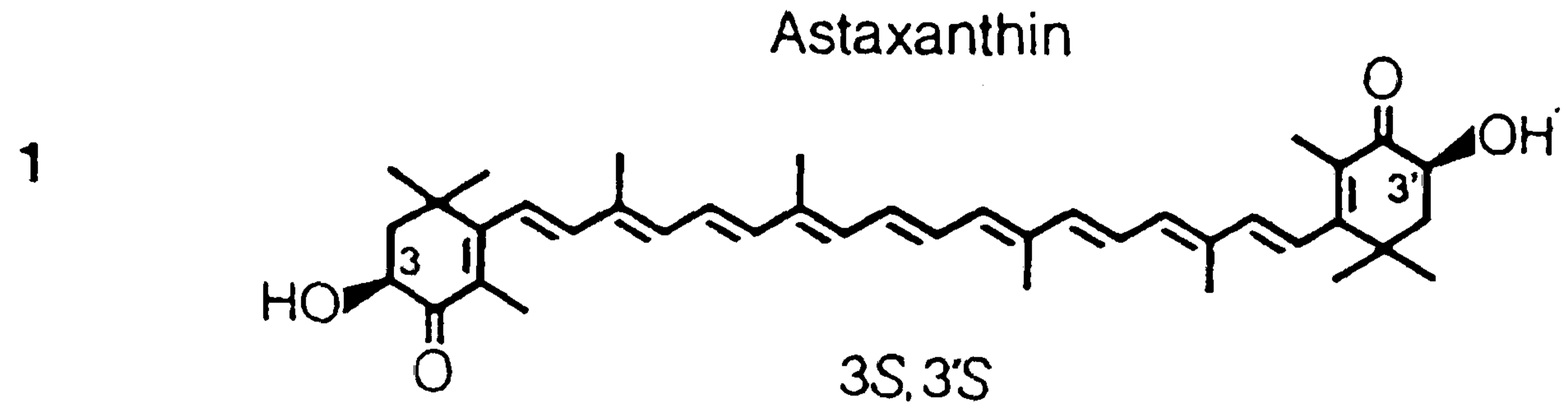

Figure 1.2: The three optical isomers of astaxanthin (1, 2 and 3) and the canthaxanthin (4) molecule (from Foss *et al.*, 1984).

Carotenoid pigments are a group of compounds which are related to carotene. They cannot be synthesised by higher animals and so come from dietary sources, especially crustaceans in the case of salmon. The pigments astaxanthin and canthaxanthin are part of a sub-group of carotenoids called the xanthophylls. They have a ketone group at each end of the molecule and astaxanthin also has two hydroxyl groups, as can be seen in figure 1.2. These give the molecules their strong anti-oxidant capabilities (Miki, 1991).

The pigments are deposited in the muscle, where they bind to the actomyosin in the myofibrils by a weak hydro-phobic bond (Henmi *et al.*, 1987). The level of pigment in the flesh can be correlated to the instrumentally measured colour of the flesh (No and Storebakken, 1991 and 1992), but colour measurement is not an accurate measure of the flesh pigment levels as many other factors affect the colour apart from these (pers. comm. O. Torrissen, Institute of Marine Research, Bergen, Norway).

The colour of the flesh can be subjectively scored using a colour card system (Colour Card for Salmonids, F. Hoffmann- La Roche Ltd., Basel, Switzerland). This is a popular method used by the industry as it gives a rapid result which can be easily interpreted. The scale for fillets runs from 11 ('light pink') to 18 ('dark red'), with scores of above 15 being required for the high quality fillets (Anon., 1995).

As the colour of the flesh is of such importance to the perceived quality of the product, the next section of this chapter reviews the work carried out so far on factors affecting the uptake of the pigments, and on how colour may be lost from the flesh post-slaughter.

1.4.6 Factors Affecting Carotenoid Uptake and Deposition

Various factors affect the uptake of pigments from the diet, including the diet formulation, the physiology of the fish and the environment. These are dietary, physiological or environmental.

1.4.6.1 Dietary Effects on Uptake of Carotenoids

Carotenoids are added to the diet of salmonids in order to obtain the 'salmon pink' colour of the flesh. The following factors affect the uptake of the carotenoids and their deposition in the muscle: type of dietary carotenoid, level of carotenoid supplement, dietary oil and dietary vitamin E.

i) Type of Dietary Carotenoid

Astaxanthin and its esters were fed to Atlantic salmon and rainbow trout and their relative rate of uptake investigated (Foss *et al.* 1984). With free astaxanthin, trout are able to utilise all three optical isomers (figure 1.2) with the same pigmenting efficacy, with no conversion from one isomer to another (which would result from epimerization at the chiral centres of the molecule).

Mori *et al.* (1989) compared the uptake and deposition of astaxanthin diesters from krill with synthesised free astaxanthin in coho salmon (*Oncorhynchus kisutch*). They found practically no difference between diester and free astaxanthin absorption and deposition, even though the esters are hydrolysed to free astaxanthin in the intestine for deposition in the flesh . This differed from the findings of Foss *et al.* (1987) who had found that the uptake of astaxanthin dipalmitate is slower than that of free astaxanthin in rainbow trout. In a diet containing free astaxanthin and canthaxanthin, the free astaxanthin is deposited in the flesh at the same rate as canthaxanthin . When astaxanthin dipalmitate was used instead of the free astaxanthin, the free astaxanthin resulting from digestion was deposited in the flesh more slowly than the canthaxanthin.

Synthetic astaxanthin and canthaxanthin were compared by Torrissen (1986). He compared the pigmentation of trout using 27ppm astaxanthin and 48ppm

canthaxanthin in the same diet. The ratio of the pigments in the diets to those deposited in the flesh indicated to the author that the astaxanthin is deposited more efficiently. However, the level of carotenoid supplementation has been shown to be important to the rate of deposition (section ii) page 28) and therefore these results are open to question. Nevertheless, *in vitro* work by Henmi *et al.* (1989) showed that adding astaxanthin and canthaxanthin solutions of the same concentration to actomyosin result in greater binding of the astaxanthin to the protein. This tended to confirm the conclusions of Torrissen (1986).

Choubert and Storebakken (1989) compared five levels of astaxanthin and canthaxanthin fed to rainbow trout. The results showed a higher retention of astaxanthin than canthaxanthin for all levels of supplementation. Foss *et al.* (1984 and 1987) also showed a difference between astaxanthin and canthaxanthin when both were added at the same level to the diet of trout, with astaxanthin giving a higher level of flesh deposition. For the same levels of flesh carotenoids they found that astaxanthin gives higher visual colour scoring than canthaxanthin (Foss *et al.* 1984). Thus astaxanthin is not only deposited in the flesh more readily than canthaxanthin, but it gives a stronger red colour to the fillet. This was supported by Skrede *et al.* (1989) using the CIELab (1976) colorimetry system, who found that at the same level of flesh pigment concentration astaxanthin gives a redder colour, as well as increased opacity and reduced whiteness compared to canthaxanthin.

A much longer experiment, starting from first feeding and adding either astaxanthin or canthaxanthin at 100ppm after 17 weeks of feeding, showed that astaxanthin initially pigmented the flesh more rapidly in trout (Bjerkeng *et al.* 1992). Later in the experiment the canthaxanthin-fed fish reached the same flesh carotenoid concentration as the astaxanthin-fed fish. This implied that over time the flesh reaches a maximum level of either carotenoid and that this level is the same for both astaxanthin and canthaxanthin.

ii) Level of Carotenoid Supplementation

If no astaxanthin or canthaxanthin is provided as a supplement in the diet then there is a gradual decrease in the carotenoid concentration in the flesh. In immature rainbow trout (initial weight approximately 500g) a significant decrease in astaxanthin concentration in the flesh occurs after 5 weeks of feeding a diet without a supplement of pigment (Torrissen, 1985). Before this point the fish keep the same concentration of flesh carotenoid, even though some growth has occurred, which suggests that the fish continue to deposit carotenoids during the first 5 weeks of receiving no dietary pigment. This is consistent with the findings of Arai *et al.* (1987) which show that astaxanthin deposition continues for 30 days after the removal of astaxanthin from the diet. After the first 5 weeks of receiving diets with no astaxanthin supplement the total amount of carotenoid in the fish drops slightly, but not significantly (Torrissen, 1985).

Choubert (1985) investigated canthaxanthin in fasted trout and in trout fed diets with no supplementary pigment. The starved trout, which stayed at the same total lipid content for the 5 weeks of the trial, remained at the same carotenoid concentration throughout. The fed trout increase their total lipids and decrease the concentration of the pigment. It was therefore concluded that, in at least the first five weeks of being fed unpigmented diets, there is little metabolism of the carotenoids from the flesh. However, it appears that dilution of the concentration would have occurred if the fish had been fed diets without pigment supplementation as the fish increase in weight.

As was discussed earlier, Torrissen (1986) showed that 27ppm of dietary astaxanthin resulted in a higher percentage retention in the flesh than 48ppm of canthaxanthin. Studies were carried out to investigate the effect of dose on pigment retention, looking either at the apparent digestion coefficients, or at pigment levels in the flesh after feeding.

The apparent digestion coefficient (ADC) is an estimate of how much of a substance is absorbed by the fish after digestion. A marker which is not digested, such as chromic oxide, is added to the feed at a known concentration. The level of the substance in the feed is also known. The feed is administered to the fish and the resulting faeces collected and analysed for the marker and for the substance. The ADC can then be calculated as follows:

$$ADC = 100 - \frac{\%\ \text{Marker in feed}}{\%\ \text{Marker in faeces}} \times \frac{\text{Concentration of substance in faeces}}{\text{Concentration of substance in feed}} \times 100\%$$

The higher the ADC, the greater the degree of digestion and uptake of the substance.

Torrissen *et al.* (1990) studied the ADC of canthaxanthin in rainbow trout. Five levels of canthaxanthin were used in diets which were otherwise identical. ADCs decreased linearly with increasing canthaxanthin in the diet, with negative ADCs resulting at the highest levels (240ppm canthaxanthin). Dissection and further examination revealed that the ADCs of canthaxanthin increased as the pigment travels through the gut. Thus the stomach has the lowest ADC for a given concentration, then the pyloric caecae, the intestine, and the hind gut which has the highest ADCs. The negative ADCs observed at high levels of supplementation were thought to be due to metabolism of the pigment in the liver into unidentified products, rather than oxidative degeneration, the products of which are known and were not found.

Following the pigment levels in the flesh is an easier way to study the effect of the level of supplementation on pigment deposition, as collection of faeces is a complicated process if it is to be carried out successfully, although this method does not allow for any metabolism of the pigment. Feeding higher levels of astaxanthin supplements resulted in higher levels of flesh pigmentation in rainbow trout and coho salmon (Sanderson and Jolly, 1994 and Smith *et al.* 1992 respectively). Increased

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dosages of canthaxanthin also increased flesh pigmentation in rainbow trout (Choubert and Storebakken, 1989).

The net retention of pigment was calculated by comparing the level of the supplement and the level of flesh pigmentation. Net retention of both astaxanthin and canthaxanthin decreases with the increasing level of supplement (Choubert and Storebakken, 1989; Torrissen, 1985), although the mean retention coefficient of astaxanthin is 1.3 times higher than canthaxanthin over five levels of dietary supplement (Choubert and Storebakken, 1989). This confirmed the conclusions of Torrissen (1986) and Henmi *et al.* (1989) that astaxanthin is deposited preferentially to canthaxanthin.

iii) Dietary Oil

With low levels of dietary oil (4% and 15% oil), trout show a significant increase in ADC of canthaxanthin with the higher level (Choubert *et al.* 1991). Torrissen *et al.* (1990) showed the same results with a greater range of oil inclusions (4.1% to 23.0%). Increasing the oil up to 35% — corresponding to the latest high oil diets — results in a higher deposition of pigment in the flesh of rainbow trout (Torrissen, 1985). Thus it was concluded that increasing the level of oil in the diet results in a greater pigmentation of the flesh at all commercially achievable levels of dietary oil.

The quality of the oil, as measured by its fatty acid complement, was also investigated (Torrissen, 1985). Different fish oils were used to make diets with different fatty acid levels. No effect of fatty acid composition of the diet was found on astaxanthin deposition.

iv) Dietary Vitamin E

A supplement of vitamin E is essential to the diet of salmonids in order to promote healthy growth as it is involved in the removal of free radicals from the body. The most commonly added form is α-tocopherol, the tocopherol most commonly found in the flesh (Frigg *et al.*, 1990; Undeland, 1995) and with a high anti-oxidant capability *in vivo* (Miki, 1991). Torrissen (1985) found no difference between the deposition of pigment in the flesh of trout fed diets with or without a supplement of vitamin E. Similarly, after 15 weeks of feeding diets with or without tocopherols, there was no effect on the level of astaxanthin in the flesh of Atlantic salmon immediately post-slaughter (Sigurgisladottir *et al.*, 1994).

1.4.6.2 Physiological Effects on Carotenoid Uptake

After ingesting the pigment, the fish must then digest, absorb and deposit it in their flesh or target tissue. This uptake could be affected by the physiological status of the fish, with factors such as size and age, sexual maturity, genetics and the number of pyloric caecae possibly being important.

i) Fish Size and Age

From hatching to first feeding the alevins contain only pigment inherited from their mother (Craik and Harvey, 1986). From first feeding, the fry have the chance to take up carotenoids, but in chum salmon (*Oncorhynchus keta*) the levels of carotenoids continue to drop (figure 1.3).

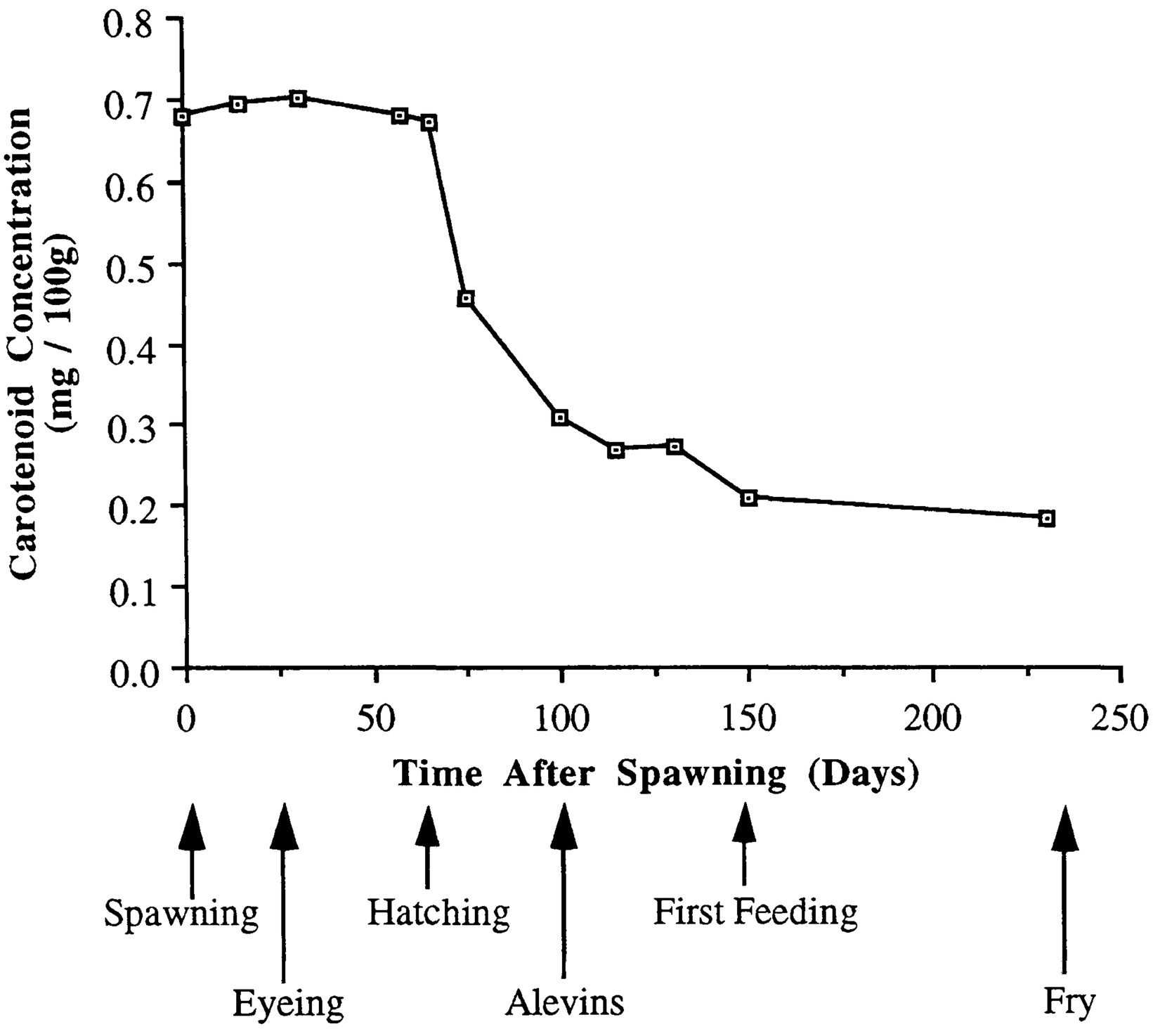

Figure 1.3: Changes in total carotenoid content from spawning to feeding fry in chum salmon *Oncorhynchus keta* (data from Kitahara, 1983).

Later the fry and parr are able to take up astaxanthin and to metabolise it, but the main deposition is in the skin (Bjerkeng *et al.* 1992). It is only after smolting that the fish change the primary target tissue for astaxanthin from the skin to the muscle. The age at smolting has been shown to have an effect on later pigment levels in the flesh (Torrissen and Naevdal, 1988). The fish which smolted after one year in freshwater attain higher pigment levels at a given time than the second year smolts. The difference in pigment levels was attributed to the difference in weight at slaughter, with the fish which smolted after one year being much larger, having had an extra year of feeding at sea.

Once in the sea the accumulation of astaxanthin in the flesh is faster than the rate of muscle increase and significant differences in flesh pigment levels arise between

salmon of different size classes (Torrissen and Naevdal, 1988). The 8-9kg class of fish had the highest flesh carotenoid concentration, but above this size class, the concentration of the pigment started to decrease. Below the 8-9kg class the concentrations were also lower, except for the 1-2kg class. This class had a slightly lower mean than the 8-9kg class, but the variation within this group was considerable. The higher levels in this class and the large variation were probably caused by the presence of grilsing fish, *i.e.* fish which were going to mature after their first summer at sea and were hence accumulating carotenoids [section ii) below]. Only some of the fish in a population would be grilse, the rest would remain immature and thus have lower levels of carotenoid in their flesh.

In rainbow trout, the age of the fish has an important effect on the level of flesh carotenoid. In their second year, fish accumulate pigments in the flesh between June and October (Sivtseva and Dubrovin, 1981). Three year old fish slowly increase their carotenoid concentrations from May to June, but flesh levels go up nine times from July to October. Four year old fish show a more uniform increase from May to October and accumulate more carotenoids than the third year fish. The authors conclude that accumulation of the carotenoids was mainly determined by age and the degree of sexual maturity.

ii) Sexual Maturity

The degree of sexual maturity has an important effect on the level of flesh carotenoids (Sivtseva and Dubrovin, 1981). Prior to maturing the fish accumulate carotenoids in their flesh. Wild salmonids start to metabolise carotenoids from their flesh as they start their spawning migration (Ando and Hatano, 1987; Kitahara, 1983). The red colour of fillets from maturing fish decreases from August onwards (Aksnes *et al.* 1986) although external signs of maturity start in May, with skin colour changes in July or early August.

Male trout lose a greater amount of pigment from the flesh (about 80%) than females (Bjerkeng *et al.* 1992), depositing most of it in their skin (Kitahara, 1983). The exact metabolites may change between species, according to the nature of the pigments found in the flesh. Kitahara (1983) suggests that zeaxanthin is one of the metabolites of astaxanthin which is found in the skin of chum salmon. This was confirmed in Atlantic salmon by Schiedt *et al.* (1988), who describe the full metabolic pathway of astaxanthin within the skin. The metabolites give the fish their characteristic reddish nuptial colours. A small amount of the pigment is also deposited in the testes and is found in the sperm (Czeczuga, 1979; Kitahara, 1983). The function of the pigment in the sperm is unknown and has not been investigated. Nor have there been any studies investigating whether the pigment has any effect on the quality of the sperm.

The females lose 20-30% of their flesh carotenoids, some of which is deposited in the skin, but most is transferred to the developing ovaries (Kitahara, 1983). The pigment is transported in the blood from the flesh to the liver. Here it may become associated with vittelogenin (Ando and Hatano, 1991). Vittelogenin is the precursor to the yolk protein vittelin, which is heavily deposited in the eggs. Thus the carotenoids are heavily deposited in the yolk protein fraction of the egg. They are also found in the oily fraction, associated with the chylomicra particles (Ando and Hatano, 1991).

Salmon farmers regard brightly coloured eggs as being of "better quality" than pale eggs (Craik, 1985). This implies that they think that the eggs will be more fertile and more likely to survive to hatching. The presence of the pigment in the eggs certainly implies that it has a function and its presence in the eggs has been found to increase their fertility and survival to hatching (Robb *et al.*, 1995). The nature of this function has been much debated. Several roles for the pigment within the egg have been suggested and these have been reviewed by Mikulin and Soin (1975), Tacon (1981) and Craik (1985). The roles include a fertilisation hormone, an oxygen attractor, a protector from light and a store for the pigment for use in the alevins after hatching.

Only the last role has been confirmed, with the alevins transferring the pigment from the yolk sac to their skin, to aid camouflage (Stevens, 1949).

After spawning the reconditioning females recover flesh pigmentation levels within four weeks (Choubert and Blanc, 1989), but a difference in the recovery rate of males has been reported: Choubert and Blanc (1989) found them slower to recover, but Torrissen and Naevdal (1988) found that the males had higher levels of pigment than the females after spawning. The explanation given for these results was that the females tend to lose carotenoids during spawning by pigmenting their eggs, but the males transfer most of their pigment to the skin, from where it is re-metabolised and returned to the flesh during reconditioning. After reconditioning, the fish attain their original flesh carotenoid levels and start to accumulate more carotenoid during the following summer spent feeding at sea ready for spawning again.

iii) Genetic Effects

The most obvious effect of genetic differences is observed between the salmonid genera. The study of four genera — *Salmo, Oncorhynchus, Salvelinus* and *Hucho* — has shown that there are not only great differences in the carotenoid complement of the different genera, but also between the species in each genus (Ando *et al.*, 1989). Within a species, differences can occur between the carotenoid complements of anadromous and river resident fish (Ando *et al.*, 1991). This may be caused by genetic differences or by the availability of different diets in the different environments.

Differences in carotenoid uptake between families of rainbow trout were investigated by Choubert *et al.* (1991), but no significant differences were found. This may have been because the families used in this trial were selected to exhibit different numbers of pyloric caecae, which may have masked other effects of family selection. When

the ability to deposit pigment in the flesh was compared between full and half sib groups of fish a significant difference was found (Torrissen and Naevdal 1984 and 1988). The authors suggest that the trait for increased pigment deposition may be selected for, but would have very low heritability, making the task of selection very hard.

iv) Pyloric Caecae

Pyloric caecae are present in the gut of the fish, where they aid the uptake of lipids. As the carotenoids are soluble in lipids and Sargent *et al.* (1979) reported that the lipids in this region were especially red, it was thought that the number of caecae might affect the rate of pigment uptake. However, an experiment selecting fish with various numbers of pyloric caecae and investigating the uptake of canthaxanthin could detect no such effect (Choubert *et al.*, 1991).

1.4.6.3 Environmental Effects on Carotenoid Uptake

The environment may also affect the uptake of the carotenoids from the diet:

i) Salinity

No and Storebakken (1992) kept rainbow trout in fresh (0 parts per thousand) or salt (32 ppt) water and fed pigmented diets for 12 weeks. When the fish were fed a standard astaxanthin pigmented diet, there was no difference in flesh and liver carotenoid content in either group. However, the skin and gut carotenoid levels were significantly higher in the seawater treatment. When canthaxanthin was used no differences in concentration were found between either the environmental or the dietary treatment groups in any of the tissues investigated. No reasons were put forward to explain this difference between the pigments.

ii) Hydrocarbon Pollutants

Paraffins, pristane and dodecylcylohexane — common oil pollutants — were added to the canthaxanthin pigmented diets fed to rainbow trout for 7 to 10 months (Luquet *et al.*, 1983). The hydrocarbons not only had a depressive effect on appetite and growth, but led to a decreased fixation of canthaxanthin in the muscle. No reasons for this decrease in fixation were suggested by the authors.

1.4.7 Slaughter of Salmon

When Atlantic salmon reach market weight, which generally ranges from 3kg to 5kg but may be higher depending on the demands of the processors, they are slaughtered. In Norway the salmon are generally crowded and then are pumped or netted from the cage into a well boat— a boat whose holds have been converted to carry live fish in water. The fish are then taken to a processing station where they are pumped or netted ashore and killed.

In Scotland fish tend to be killed on site. The nets are drawn up around the fish to crowd them and make them easier to catch. They are then pumped or netted out and killed. During the crowding, which may last several hours (pers. obs., 1997), the fish show increased swimming activity and some thrashing around at the surface.

Commercial slaughter in the United Kingdom at present is carried out by exsanguination following the use one of two methods — carbon dioxide anaesthesia or percussion stunning (Anon., 1995) — which are described below:

i) Carbon Dioxide Anaesthesia

Ideally a tank of sea water has carbon dioxide gas bubbled through it using fine gas diffusers. When the pH of the water is below 4.5, the water is taken to be saturated with the gas (Anon., 1995). The fish are then netted into the tank where they are left for at least six minutes, or until they show no signs of response to external stimuli such as fin pinches. At this stage the fish are lifted out of the anaesthetic tank and the gills on both sides of the head are cut using a sharp knife before the fish are placed in a bin of ice slurry to bleed out (exsanguinate).

In reality, when the fish are added to the anaesthetic bath they show an immediate reaction to the carbon dioxide. This usually involves rapid swimming and repeated escape attempts. Whether this is a reaction to the rapid anaesthesia, or a specific aversive reaction to carbon dioxide is not known. Kestin *et al.* (1995a) showed that fish reacted less violently to both an acid bath of the same pH as that of the carbon dioxide saturated water and anoxic water than they did to carbon dioxide anaesthesia. Erikson *et al.* (1997) judged this method of slaughter to exacerbate the stress to the fish at slaughter.

The pressures of time tend to cause the slaughter process to be rushed and the fish may receive gill cuts before they are fully anaesthetised (pers. obs., 1996). Kestin *et al.* (1995a) showed that under ideal conditions the fish stopped moving after about two minutes, but remained sensible to external stimuli for four minutes after immersion in the carbon dioxide anaesthesia bath. Many slaughter teams use the cessation of movement as the cue to start exsanguination, which may result in the fish being sensible to the process of exsanguination.

ii) Percussion Stunning

Commercially the fish are netted or pumped from the cage on to a large table, where they receive a blow to the top of the head using a short wooden or polypropylene club (priest). If sufficient force is applied, this causes the skull to accelerate with the brain following momentarily later, resulting in shearing forces within the brain (Wotton, 1997). There is a threshold of force below which the recovery of a stunned fish is possible. Above this threshold there is no recovery (Kestin *et al.* 1995a). After the stun the gills of the fish are slit and the fish placed in bins of ice slurry to bleed out. This ensures the eventual death of the fish even if the blows are below the threshold needed to achieve irrecoverable insensibility — *i.e.* death.

As the result of the instantaneous stun, providing the blow is applied correctly, this method appears less stressful to the fish than carbon dioxide anaesthesia when carried out under ideal conditions (Erikson *et al*, 1997). However, under commercial conditions when there are large numbers of fish to be killed by hand, the accuracy of the blows may vary, especially towards the end of the day. A misplaced blow results in violent aversive reactions from the fish, which obviously try to escape (pers obs., 1996). Also the fish show some reaction to their removal from water when they are placed on the killing table. However, the time from their arrival on the table to receiving a stun is generally very short — 2 to 6 seconds — (pers. obs., 1996), so the reaction is minimal.

Both methods of slaughter cause some form of stress to the fish. The absolute level of stress is debatable, but what is irrefutable is that the fish display a high degree of activity at slaughter, involving thrashing around and some escape attempts. The high level of activity is also observed in the crowding procedure prior to slaughter.

1.4.8 Effects of High Pre-Slaughter Activity on Mammal Flesh Quality

In red meat species a link between stress at slaughter and poor flesh colour and texture has been shown (reviewed by Warriss, 1996). Increased stress at slaughter results in pale, soft flesh, with a high loss of exudate from the flesh post-slaughter. This condition is described as PSE (pale, soft, exudative).

The condition arises as a direct consequence of the stress prior to slaughter. Under stress or conditions of high physical activity the animals' demand for oxygen increases. The demand for oxygen may even exceed the supply, resulting in anaerobic respiration before slaughter. Even if the respiration remains aerobic, the level of available oxygen within the body is decreased. When the stress is removed and the animal allowed to recover normally the oxygen level is able to build up again. This also allows the clearance of the by-products of anaerobic respiration from the cells.

From the point of slaughter there is no further transport of oxygen within the body and the cells are left with the oxygen supplies that they had at the point of death. When these supplies are used up the cells enter anaerobic respiration. The time to the point when anaerobic respiration occurs after death is controlled by the level of oxygen at slaughter, which in turn is affected by the level of pre-slaughter stress as has been described above.

During anaerobic respiration lactic acid is produced from glycogen, instead of the acetyl-coenzyme A complex which is formed with aerobic respiration (figure 1.4). As the level of acid builds up, the enzymes within the flesh are denatured, preventing further reactions. The enzymes affected include those involved in respiration, thus leading to its cessation. After this point the pH of the flesh remains constant.

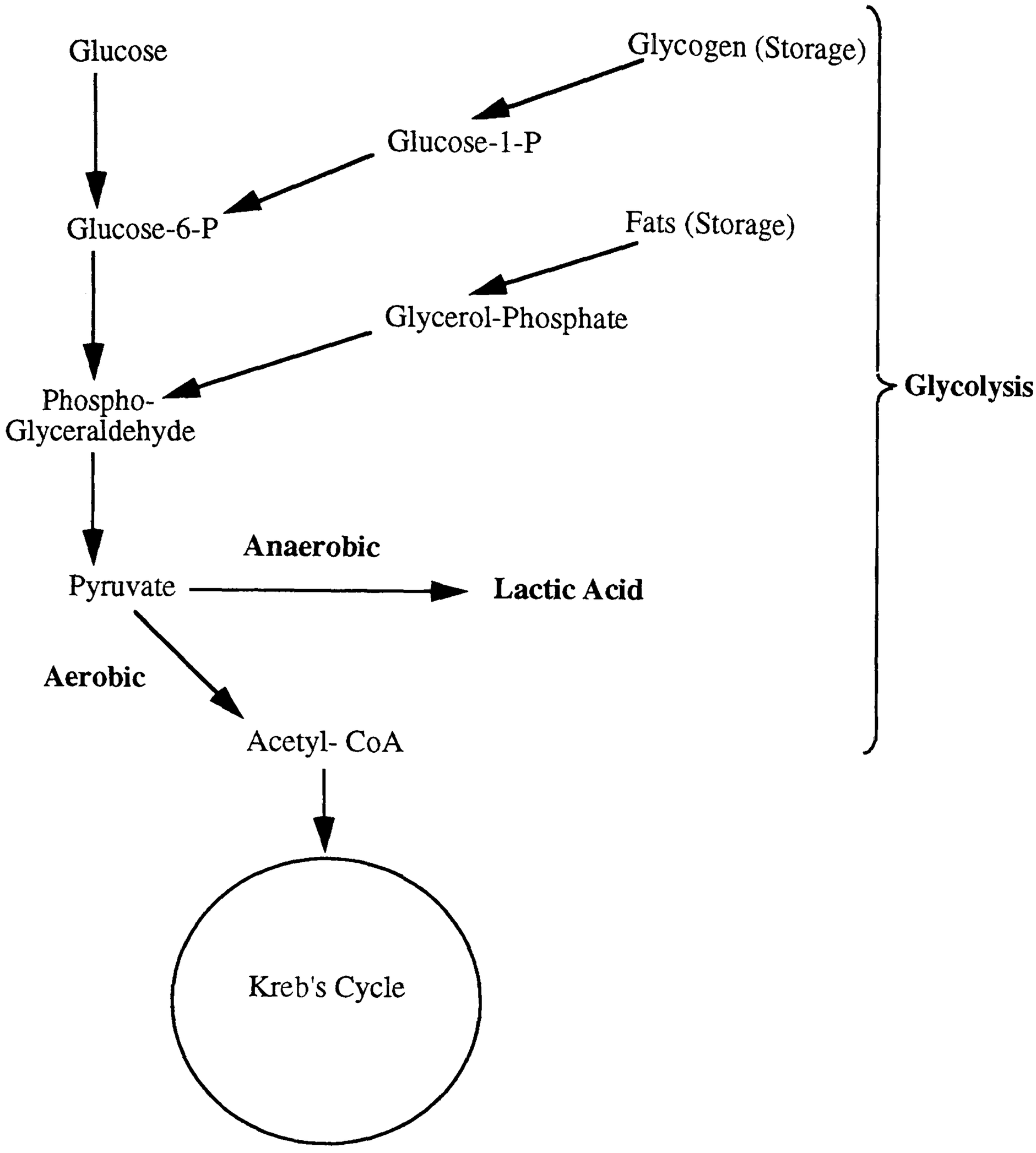

Figure 1.4 Simplified flow chart of glycolysis and the Kreb's Cycle. After Dorit *et al.* (1991)

However, the cessation of respiration also prevents the production of adenosine triphosphate (ATP), used for energy within the cells. One of its functions is to allow the muscle myofilaments to slide over each other. The loss of the ATP supply causes the stores of ATP to be used up and so the myofilaments are no longer able to slide. Thus *rigor mortis* sets in.

Rigor occurs in all animals, stressed or unstressed, as the enzymes are particularly sensitive to pH for their reactions or run out of substrate and so are unable to synthesise ATP, but the time to rigor onset is determined either by the speed of acidification of the flesh or by the time taken to use up all of the substrate. The rate of acidification is affected in turn by the time to the onset of anaerobic respiration, which is governed by the level of stress pre-slaughter as has been discussed above.

The rate of acidification of the flesh also affects proteins other than enzymes. Rapid acidification causes some denaturation of the soluble proteins within the flesh. They become insoluble and release the water they are associated with; the excess water within the flesh is lost as exudate. As has been explained, the flesh of stressed animals acidifies faster and so it produces more exudate, hence the high level of exudate associated with PSE in pigs. The loss of water and change in protein structure also affects the texture of the flesh, so the flesh of stressed animals becomes more spongy to the touch — hence the softness of PSE.

Finally, the colour of the flesh is altered. The level of pigments within the flesh of stressed and unstressed animals remain the same after slaughter, but the colour of flesh from stressed animals can be paler (Warriss, 1996). The changes in protein structure discussed above result in the interference of the light reflected from the surface of the muscle. In an unstressed animal the structure of the muscle is homogenous, so the incident light is reflected from the surface with very little interference. The loss of water from the protein in the muscle of the stressed animals, caused by their denaturation, increases the refraction of light at the water protein interface. The resulting interference causes the reflected light to appear whiter than that reflected from the unstressed flesh. Thus the paleness of PSE is not a result of a loss of pigment.

1.4.9 Effects of Activity Pre-Slaughter on Fish Flesh Quality

The musculature of fish differs greatly from that of mammals. In salmonids most of the edible portion consists of white muscle, with a few red muscle fibres within this portion (figure 1.5). Greater numbers of red muscle fibres run along the flanks and the dorsal ridge. The red muscle is used for normal swimming, whereas the white muscle is used for rapid bursts of activity and to some extent for routine swimming (Driedzic and Hochachka, 1978). The greater the degree of activity, the greater the use of the white muscle.

The respiration of the red muscle is predominantly aerobic while that of the white muscle is largely anaerobic as it is poorly vascularised (Driedzic and Hochachka, 1978). The end products of both aerobic and anaerobic metabolism in fish are similar to those of mammals discussed above. Thus during anaerobic metabolism lactate is produced which causes a reduction in the pH of the cells and the poor vascularisation of the white muscle does not allow its rapid clearance.

As white muscle use increases with increasing exercise and the white muscle mainly respires anaerobically, increasing the level of exercise lowers the pH of the muscle. If fish are killed immediately after exercise, or stress resulting in rapid movement, the flesh has a lower pH as is demonstrated below. This could make it more susceptible to a PSE-like condition.

The effects of activity at slaughter have been investigated in several species of fish. Lowe *et al.* (1993) showed that in the snapper (*Pagrus auratus*) there are effects on blood and muscle chemistry caused by activity prior to death. The fish were either 'rested' in aquaria before slaughter or were chased for one hour around the tanks and then killed. The higher activity group showed a significantly higher blood cortisol

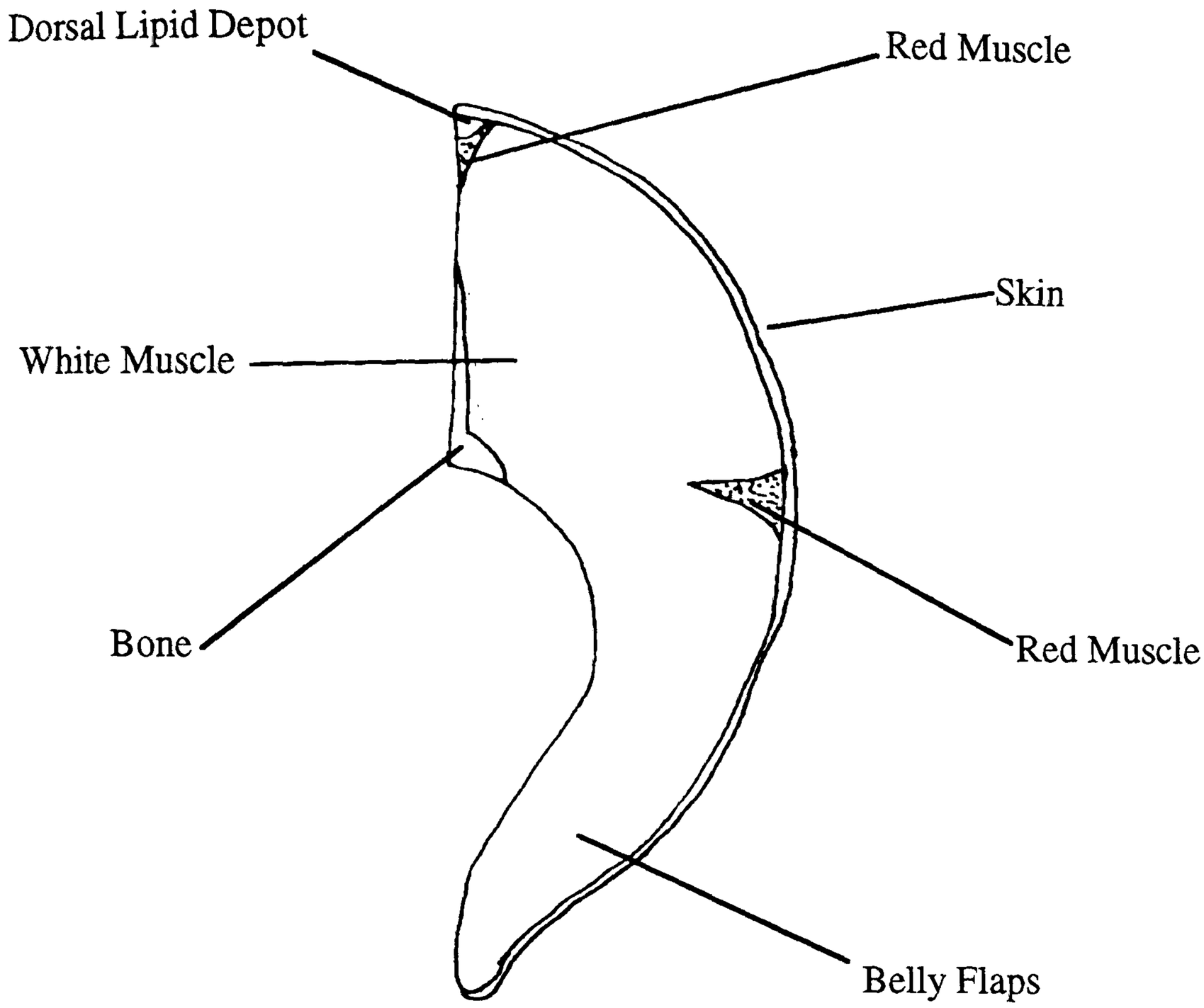

Figure 1.5 Distribution of red and white muscle within the flesh as seen through a cross-section of a salmon fillet taken below the dorsal fin.

level ($p<0.05$) at death. This indicated that a higher level of stress had been experienced by the fish.

Chasing Atlantic salmon for one hour prior to slaughter results in a significant ($p<0.05$) loss of phosphocreatine (PCr) and ATP from the white muscle, compared to rested fish (Erikson *et al.*, 1997). The exercised fish also show a significantly higher ($p<0.05$) level of inosine monophosphate (IMP). This indicates that the exercised fish use more energy than the rested fish during the period prior to slaughter. This finding supports the results of Lowe *et al.* (1993) which showed that the 'rested' snappers have higher ATP levels at slaughter than the exercised group immediately post-slaughter. Figure 1.6 shows how the compounds are related in the production of energy for the muscles *in vivo*.

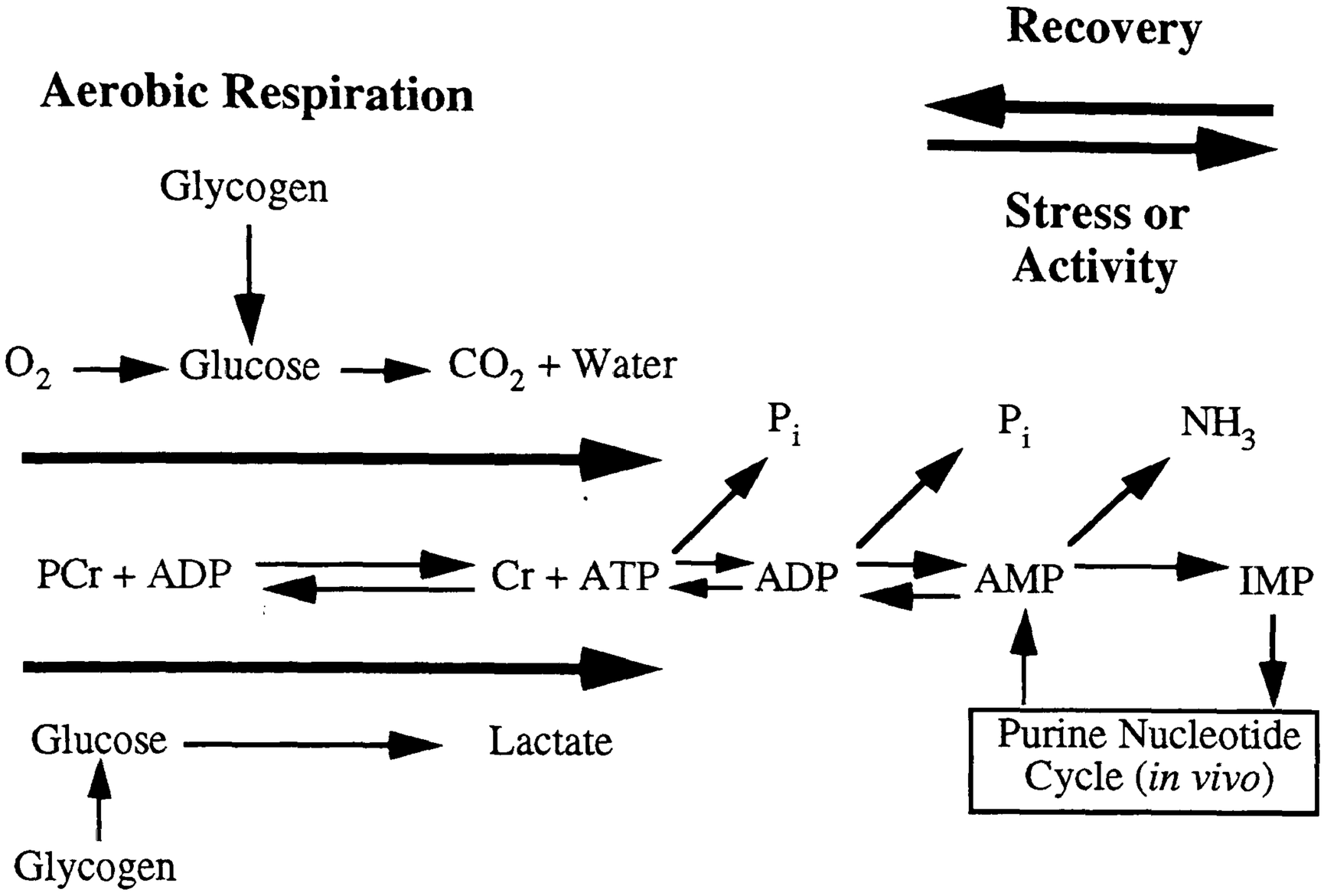

Figure 1.6 Energy metabolism in fish white muscle during rest (aerobic), stress or activity (anaerobic) and recovery (after Erikson, 1997). PCr — phosphocreatine, Cr — creatine, ATP — adenosine triphosphate, ADP — adenosine diphosphate, AMP — adenosine monophosphate, IMP — inosine monophosphate, P_i — inorganic phosphate.

The K-value of a fish is a measure of the *freshness*, with lower K-values indicating greater *freshness* (Saito *et al.*, 1959). It is defined as:

$$\text{K-value} = \frac{\text{HxR + Hx}}{\text{ATP + ADP + AMP + IMP + HxR + Hx}} * 100\%$$

where HxR is inosine and Hx is hypoxanthine, which are breakdown products of IMP during post-slaughter storage and are thus regarded as signs of loss of *freshness*. Hx also gives a bitter flavour which is disliked by taste panels (Erikson, 1997). The effects of the reduced levels of PCr and ATP and increased IMP immediately after slaughter in the exercised group lead to increased K-values of the fish during storage

post-slaughter (Erikson *et al.*, 1997; Lowe *et al.*, 1993). Therefore, it could be concluded that the exercised fish lost *freshness* more rapidly than the 'rested' fish.

The rested fish use aerobic respiration during the pre-slaughter period; post-slaughter the remaining oxygen in the muscles is slowly used up, before the cells start to respire anaerobically. In contrast, the exercised fish use anaerobic metabolism to obtain energy during the pre-slaughter period. This results in increased lactate being recorded in the exercised snapper (Lowe *et al.*, 1993) and in rainbow trout killed using carbon dioxide anaesthesia (Azam *et al.*, 1989), which resulted in these fish having lower muscle pH values than the control groups of 'rested' or 'unstressed' fish in these experiments.

The decrease in pH leads to the interruption of the synthesis of ATP by the muscles, a process which continues for a limited period post-mortem while the cells continue respiration. With the cessation of ATP synthesis, the remaining ATP within the cells is used up during metabolism. When the ATP in a muscle cell is depleted the cell enters rigor, as the myofibrils within the cells are no longer able to slide over each other. As more individual cells enter rigor the whole body becomes less flexible. This can be measured by the degree to which the body of the fish is able to bend (Iwamoto *et al.*, 1987; Azam *et al.*, 1990). Increasing activity prior to slaughter reduces the concentration of ATP and the pH of the muscle, as shown above in the work of Erikson (1997) and Lowe *et al.* (1993). It could therefore be expected that the more exercised fish would enter *rigor mortis* earlier than 'rested' fish. This has been shown by Lowe *et al.* (1993) in snapper, Azam *et al.* (1990) in rainbow trout and Berg *et al.* (1997) in Atlantic salmon.

1.4.9.1 Effects of High Activity / Stress on Texture

Jerrett *et al.* (1996) investigated the effect of stress on the texture of the flesh of chinook salmon (*Oncorhynchus tshawyttscha*) using a low voltage electrical stimulator to create a model system to mimic the effects of stress. The application of a low voltage alternating current along the length of the body immediately after slaughter stimulates the muscles, causing their rapid contraction and relaxation, similar to that observed in periods of severe stress. Unstressed fish were obtained by anaesthetising the fish with the minimum disturbance. When the fish had been fully anaesthetised they were removed from the water and killed.

Jerrett *et al.* (1996) found that the muscles of the stressed group were more prone to breaking than those of the unstressed animals. This implies that there are effects of stress on the proteins. The authors also noticed that the flesh of the unstressed animals appeared more translucent than that of the stressed animals, although this was not measured (pers. comm. J. Holland, Food and Crop Research, New Zealand).

1.4.9.2 Effects of High Activity / Stress on Colour

The colour of the flesh of salmonids is affected by a great many factors, some of which have been discussed above. However, even when these factors are controlled, a difference in colour between two batches of salmon may be found (pers. comm. C. Errard, Pinney's of Scotland, Brydekirk, Dumfriesshire, Scotland). This difference is too large to be explained by natural variation, so it is apparent that another factor is important in the colouration of the fish.

If the salmon are harvested during bad weather, there is a greater likelihood of their having poorly coloured flesh (pers. comm. A. Brown, Hydro Seafoods, South Shian, Scotland). Further, the incidence of gaping within the flesh also increases. Gaping is

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a condition which results in the disintegration of tissues between, and in extreme cases within, the myotoma. The flesh may completely disintegrate on filleting in some cases, or be so badly damaged as to be useless for further processing such as smoking (pers. comm., A. Dingwall, Pinneys of Scotland, Brydekirk, Dumfriesshire, Scotland). Therefore it seems likely that there is a direct link between stress at slaughter and poor flesh colour and texture.

1.4.10 Post-slaughter Handling and Storage

After slaughter the fish are eviscerated and chilled on ice until rigor has resolved. They are then processed (filleted, steaked, frozen or smoked) and subjected to some form of storage until they are bought and eaten by the consumer. During this processing and storage period the fish are liable to deteriorate (Connell, 1995).

Fresh fish may be left in the round or cut into steaks. The whole fish may be stored for up to 12 days after slaughter on ice (pers. comm., P. Whitlock, Marks and Spencers, London). Alternatively the fish can be frozen and stored for much longer periods, although the storage temperature is crucial to the quality of the fish (Connell, 1995). For instance, in the dogfish *Squalus acanthias* a temperature of -30˚C was considered to give twice the storage period of a temperature of -18˚C (Bilinski *et al.*, 1980).

Smoked fish fillets are usually vacuum packed whole or sliced and can be kept in refridgerated storage at 4˚C for up to four weeks (pers. comm., C. Errard). During storage the product is generally kept on an illuminated chilled display shelf until sale.

1.4.11 Keeping Quality

During storage post-slaughter, the flesh of the fish deteriorates in quality. The rate of deterioration depends on the storage conditions (Connell, 1995). The loss of eating quality is caused primarily by the saturation of the lipids within the carcass (Johansson and Kiessling, 1991) and the increase of other off-flavour-causing compounds such as hypoxanthine, a break down product of IMP which is itself a flavour enhancer (Erikson, 1997). Both result in the reduction of the perceived 'freshness' of the fish. Deterioration also occurs following the build up of bacteria on the flesh, producing off flavours and even toxins (reviewed by Connell, 1995).

The rate of saturation of the lipids can be slowed by antioxidants within the flesh. Natural antioxidants found in the flesh of the fish include tocopherols (vitamin E), vitamin C and carotenoid pigments such as astaxanthin (reviewed by Undeland, 1995). Tocopherols have very strong antioxidant capabilities (Miki, 1991) and experiments have shown that increasing the levels of tocopherols in the flesh by increasing dietary levels results in increased stability of the lipids in the flesh post-slaughter (Frigg, *et al.*, 1990). Sigurgisladottir *et al.* (1994) found no effect of the level of tocopherols in the flesh of the fillets of Atlantic salmon either on the taste or the texture of the fish. However, in this trial only three fish per diet were used and the high degree of natural variation between individual salmon makes this result open to criticism.

1.4.12 Lipid Oxidation During Storage

There are high levels of unsaturated fatty acids in the flesh, which are susceptible to oxidation. An increase in the TBA (thiobarbituric acid) reaction levels observed during storage indicates that their oxidation occurs (Tomás and Añón, 1990; Silva *et al.*, 1994 and Bjerkeng and Johnsen, 1995), causing the occurrence of rancid flavours

(Waagbo *et al.*, 1993). The oxidation requires activation either by light (Bjerkeng and Johnsen, 1995) or by enzymes (German and Kinsella, 1985), even though the reaction of oxygen and the unsaturated lipids is thermodynamically favourable. The skin of rainbow trout has shown lipoxygenase activity, which would initiate this reaction (German and Kinsella, 1985). The display conditions of fillets or steaks result in the flesh being exposed to light, which may also initiate the oxidation observed by Bjerkeng and Johnsen (1995).

Once lipid oxidation has been initiated, free radicals are generated which result in the propagation of the reaction (figure 1.7). The reaction can only be stopped by two free radicals reacting together or the reaction of a free radical with some form of inhibitor, of which true antioxidants are a subgroup (Undeland, 1995). α- Tocopherol and carotenoids, including astaxanthin, are included in the true antioxidant categories and as such may improve shelf-life. The addition of α- tocopherol to the diet of red meat species has been shown to lead to increased stability of the pigment oxymyoglobin in the flesh during post-slaughter storage (Wood and Enser, 1997). This is caused by the decrease in the rate of its oxidation to metmyoglobin.

1.4.13 Loss of Vitamin E

During the frozen storage of smoked salmon Ackman and Timmins (1995) showed a decrease in the amount of vitamin E in the flesh, whilst the peroxide value increases. This indicates that vitamin E is used up in the oxidation process. Increasing the level of vitamin E in the fillet of channel catfish (*Ictalurus punctatus*) results in a reduced rate of oxidation (O'Keefe and Noble, 1978), as the vitamin has strong antioxidant properties (Miki, 1991). However, at high levels of fillet lipid, the oxidation rate is so great that it may not be affected by increasing the level of the vitamin (Silva *et al.*, 1994).

Initiation	$X\bullet + RH \rightarrow R\bullet + XH$
Propagation	$R\bullet + O_2 \rightarrow ROO\bullet$
	$ROO\bullet + RH \rightarrow ROOH + R\bullet$
	$RO\bullet + RH \rightarrow ROH + R\bullet$
Termination	$2R\bullet \rightarrow R\text{-}R$
	$R\bullet + ROO\bullet \rightarrow ROOR$
	$2ROO\bullet \rightarrow ROOR + O_2$
	$2RO\bullet + 2ROO\bullet \rightarrow 2ROOR + O_2$
	$ROO\bullet + InH \rightarrow ROOH + In\bullet$
	$RO\bullet + InH \rightarrow ROH + In\bullet$

Figure 1.7: Auto-oxidation initiation, propagation and termination of organic molecules. X• is the initiating molecule causing the oxidation of the organic molecule RH. The symbol • indicates a free radical. InH is an inhibiting molecule such as an antioxidant. The free radical In• does not have sufficient energy to cause further oxidation (after Undeland, 1995).

Although α-tocopherol has one of the highest antioxidant capabilities of the natural antioxidants *in vivo*, in the flesh post-slaughter its properties are weakened (Belitz and Grosch, 1987). In comparison ∂-tocopherol and λ-tocopherol show weak biological activity, but are superior to α-tocopherol in the flesh post-mortem (Juillet, 1975). This is partly because α-tocopherol is easily oxidised by atmospheric oxygen, which the flesh is exposed to, while the other forms are more stable, and partly because α-tocopherol in the flesh forms two different products on reaction with free radicals, one of which is itself a radical which can initiate autoxidation (Belitz and Grosch, 1987). Therefore the α-tocopherol has less of an oxidation-inhibiting effect in the flesh than might be expected.

1.4.14 Loss of Carotenoids

Carotenoids are also used up during lipid oxidation. Ingemansson *et al.* (1993) and Sigurgisladottir *et al.* (1994) showed that astaxanthin and vitamin E levels decrease in the flesh of frozen salmon. However, both report no effect of astaxanthin on the lipid stability, which implies that the rate of oxidation was greater than the level of the carotenoid in the flesh could control. This was contradicted by the work of Bjerkeng and Johnsen (1995) who found that higher levels of astaxanthin in the flesh result in the oxidation rate being reduced. Bjerkeng and Johnsen (1995) used fish with higher levels of the carotenoid in the flesh and this may have accounted for the difference in results.

The oxidation of the carotenoids results in the production of colourless compounds (figure 1.8). Thus the flesh tends to lose colour during storage as the carotenoid is oxidised.

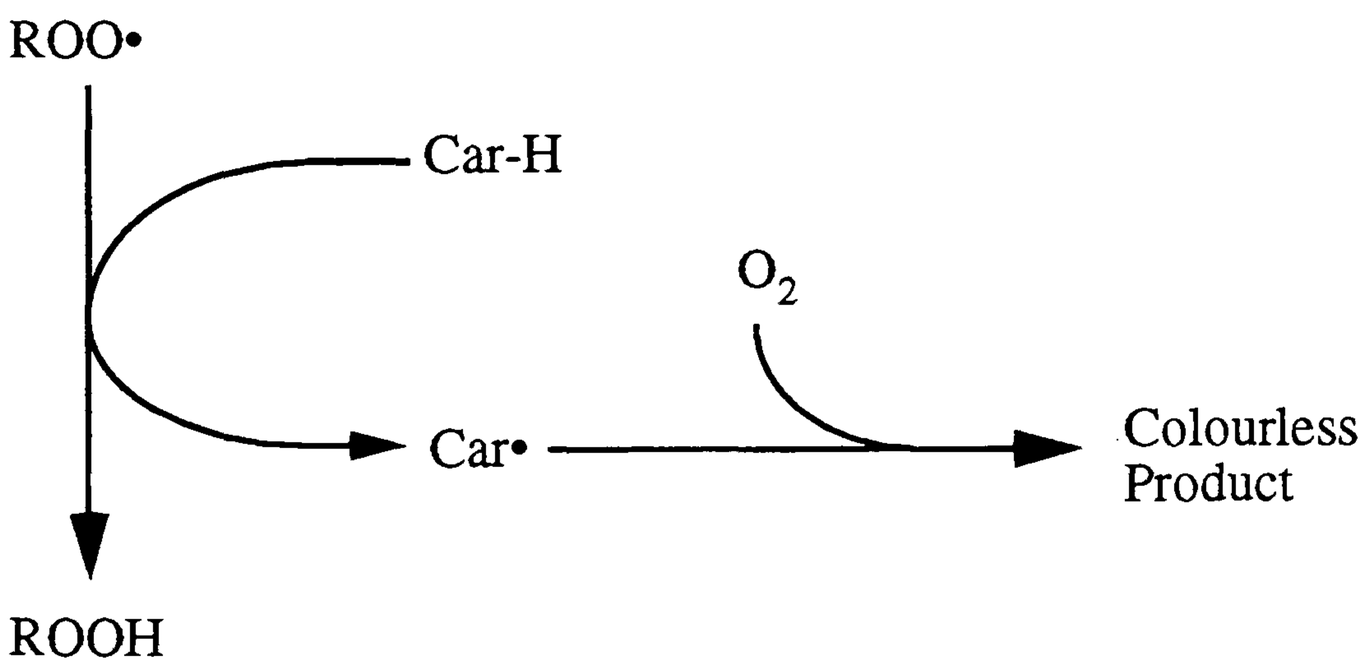

Figure 1.8: Antioxidant reaction of carotenoids (Weber and Grosch, 1976). Car indicates the carotenoid molecule and O_2 an oxidising molecule, not necessarily atmospheric oxygen.

Tsukuda and Amano (1968) linked the effect of skin enzymes with the decrease of the carotenoids. They had previously reported that the skins of red fish such as the gurnard (*Chelidonichthys kumu*) show a loss in colour during storage on ice (Tsukuda

and Amano, 1966). This was caused by the activity of a substance in the skin of the fish reacting with the carotenoids there to produce colourless compounds (Tsukuda and Amano, 1967). In a series of experiments they then showed that this was most likely to be caused by the activity of a lipoxidase in the skin (Tsukuda and Amano, 1968).

The loss of carotenoids and vitamin E from the flesh of salmonids may be related to the presence of such an enzyme. The presence of the lipoxygenase enzyme in the skin has been shown in rainbow trout (German and Kinsella, 1985). During the processing of the fish, the skin of one fillet often comes into contact with the flesh of another, which would allow the enzyme to initiate the oxidation reaction. Propagation of the reaction would continue until an antioxidant terminates the reaction. In the case of vitamin E and astaxanthin, the reaction with the radical is irreversible and so the antioxidants are effectively lost from the flesh.

1.4.15 Eating Quality

Following the storage period, the product is finally consumed. This is the single most important part of the whole process and clearly the eating quality of the product will help to determine whether the consumer makes a repeat purchase.

The eating quality of food products is best assessed using a taste panel. Taste panels may take several forms, each with their own strengths and weaknesses (reviewed by Nute, 1996). One particular method, sensory profiling, allows the taste panellists to rate the samples from each experimental group for a series of texture and flavour attributes which can be determined by the panellists in a training session. This method gives detailed information on the samples, providing that the experiment is carefully designed.

The texture attributes can be split up into several related groups, depending on when they are determined (Brandt *et al.*, 1963). For instance, the attributes may be sensed on the first bite of the sample, on chewing and on assimilation of the chewed sample prior to swallowing. The texture at each point can be further subdivided into mechanical (such as *chewy*) and geometrical or mouth-feel (such as *oily*) properties of the sample (Brandt *et al.*, 1963). This methodology has been used to evaluate the texture of various fish species (Cardello *et al.*, 1982). Similarly, flavour profiles can be developed, although the flavours are only judged during chewing (Cairncross and Sjöström, 1950). Such profiling methods have been used on salmonids (for example Ostrander and Martinsen, 1976; Johansson and Kiessling, 1991; Kestin *et al.*, 1995b).

Sensory profiling has allowed many important factors affecting flavour and texture to be determined. These factors occur throughout the life and during the post-mortem slaughter of the fish. As has been discussed in section 1.4.1.2, the diets may affect the proximate composition of the fish. The level of flesh lipid is shown to have strong effects on the flavour and texture of cooked rainbow trout (Kestin *et al.*, 1995b). Texture, odour and flavour of rainbow trout are also significantly affected by pre-slaughter starvation (Johansson and Kiessling, 1991; Wiseman, 1993). Such starvation is common industrial practice in both salmon and trout farming. Finally the post-slaughter storage has a large effect on the perceived texture and flavour. Both the storage temperature and duration are important to determining the eating quality of the flesh in rainbow trout. A longer storage period results in less perceived *freshness*, as does warmer storage conditions (Johansson and Kiessling, 1991).

The importance of the level of flesh lipid on the eating quality of smoked or cooked salmon has not been described in the literature. If it is as important as it was found to be in cooked rainbow trout (Kestin *et al.*, 1995b), then flesh lipid should be controlled for future eating quality experiments. Further, with the susceptibility of the flesh lipids to oxidation and hence rancidity, it is important that the storage conditions for

all samples for taste panelling be treated in the same way if any *in vivo* effects are to be determined.

The susceptibility of the lipids to oxidation during storage post-slaughter raises the question of the importance of flesh anti-oxidants in preserving eating quality. The effects of such anti-oxidants on the lipids has been discussed above, but there is no literature on the effect that they may have on the eating quality. It may be expected that increasing the level of anti-oxidant in the flesh would slow the onset of rancid flavours, which have been shown to develop with storage (Johansson and Kiessling, 1991). However, increasing the levels of the anti-oxidants may adversely affect the eating quality in the fresh fish. For instance, ascorbic acid was used as a top dressing on salmon steaks to try to prevent oxidation. This resulted in a strong lemony flavour which was not always appreciated by the consumer (pers. comm. C. Errard), emphasising the need for eating quality assessments on material from trials.

1.5 Conclusions

The review of literature shows that a lot of work has been carried out into the factors affecting the quality of fish flesh. However, some of the works are contradictory and some of the earlier experiments on diets may now be irrelevant to the modern diets, owing to the much higher levels of oil used currently. Some areas have been completely under-researched — or the research carried out is locked into commercially confidential reports. These areas include factors affecting eating quality and the effects of pre-slaughter handling and slaughter methods on the flesh quality.

Personal communication with members of the salmon farming and processing industries has highlighted where research would be particularly valuable. The pigmentation of the flesh of the fish is an especially important area for research due to the cost of feeding the dietary pigments.

With the introduction of the high oil diets, the effects of increasing dietary oil on the composition of the flesh are also important for the industry. It is essential to understand how the changing levels of lipid in the flesh may affect its eating quality, in order to obtain a high over-all level of quality in the products. Current knowledge of factors affecting eating quality in fish is sparse, mainly owing to the expense of carrying out trials designed to determine such effects, and there is an obvious need to redress this deficiency.

Finally the effects of pre-slaughter handling and slaughter techniques on the flesh quality of the fish are areas that obviously requires in-depth studies. However, it is also apparent that as so little information is presently available on this subject that a broad ranging overview of the whole subject should be carried out to provide much more information about what is happening at the slaughter period.

Chapter 2

The Effect of Dietary Oil on the Proximate Composition of Fillets and Viscera

2.1 Introduction

Shearer (1994) reviewed some factors affecting the proximate composition of fish. Although there has been previous work on factors affecting the proximate composition of fish, much of it has been carried out on species other than salmonids (for example Garling and Wilson, 1976; Winfree and Stickney, 1981) or on young salmonids (for example Brett *et al.*, 1969; Reinitz, 1983). This was probably in part due to the expense of carrying out a trial on market size salmonids. However, as was observed in chapter one, salmonids go through two distinct growth patterns in their lifecycle. Differences in the deposition of protein and lipid in the body also occur at these times, so the extrapolation of data from fry to large salmonids may not be valid. This is especially noticeable for flesh protein content where the relation between protein content and live weight changes for fish above 100g (Shearer *et al.*, 1994).

In Shearer's review (1994), the fact that little of the work was recent was highlighted, for example Brett *et al.* (1969), Garling and Wilson (1976), Reinitz (1983) and Staples and Nomura (1976) were papers often cited. These earlier works have been outdated by the production of new higher-oil diets which contain much greater levels of oil than were previously achievable. It is accepted that such an increase in the level of oil will result in faster growth, but there is concern that the level of lipid in the flesh will become unacceptably high if greater and greater levels of dietary oil are used.

The work in this chapter aims at investigating the effect of the levels of dietary oil on the proximate composition of the fillets and viscera of salmonids at market size. Such an effect can be investigated in two ways; either by using a large number of diets with different oil levels and performing a regression analysis of fish growth against oil level, or by using two diets of different oil levels and feeding them to several tanks of fish and comparing the mean weights of the fish from each tank. The first type of trial

requires many different diets to be produced in order to have enough data points on the graph to perform the regression analysis and to minimise the effects of any outliers on the results (Zolman, 1993). It should be remembered that for such a trial the sample number, *n*, is always equal to the number of tanks used, not the number of fish, as the fish are not statistically independent owing to interactions within each tank (Zolman, 1993). The results of such a trial would give a great deal of information on the levels of oil required in the diets of the fish to give the best growth rates and the optimal proximate composition.

However, the optimal proximate composition of the fish is not yet known. Therefore, it was decided to investigate whether dietary oil *per se* had an effect on the proximate composition of the fish. This was best done by using two diets of different oil levels and feeding them to several tanks of fish. A minimum number of tanks for each treatment is thought to be 3 for survival studies (pers. comm., Kjersti Gravingen, Apothekernes Laboratorium A.S.). However, for studies on dietary effects a larger number should be used, owing to the high degree of natural variation within the fish. As with the regression type of trial, *n* is always equal to the number of tanks in each treatment group, not the total number of fish in each treatment when investigating the effects of the diets on growth (Zolman, 1993). As can be seen, this experimental design requires large numbers of fish and so, for reasons of cost, rainbow trout are used as a model species in this study rather than Atlantic salmon. Rainbow trout are a similar species to Atlantic salmon, both in their life-cycle and in their flesh quality, thus making them a suitable model species for such a trial. However, it should be noted that the diets of rainbow trout have a much lower oil level than salmon diets. Modern trout diets are approximately 25% oil compared to the salmon diets of approximately 32% as discussed in the introduction to this thesis. The other basic ingredients of the diets are the same as salmon.

2.2 Method

2.2.1 Diets

Two diets were formulated for this experiment (table 2.2.1). Both diets were designed to include the same protein levels (they were isonitrogenous), using the same fish meal. Different levels of fish oil were added from the same stock to achieve two different levels of oil after extrusion. The diets were made by EWOS Technology Centre (Livingstone, U.K.) immediately prior to the trial and were stored in a cool (approximately 10°C) feed store during the trial to keep them fresh. The diets were extruded through a die to cut them at 4mm diameter, which was the recommended size for the fish used (Appendix 1).

Table 2.2.1 Composition of the diets after extrusion (analyses by Niall MacDonald, EWOS Technology Centre, Livingstone, United Kingdom according to AOAC methods, 1991). Basic diet ingredients were LT fishmeal, fish oil, wheat, anti-oxidant (BHT), vitamin and mineral supplements and astaxanthin.

	Low Oil Diet	High Oil Diet
Oil (%)	16.2	26.4
Protein (%)	49.2	47.6
Moisture (%)	6.3	6.0

2.2.2 Fish

A population of approximately 1,000 all-female rainbow trout was raised in the same tank (7m diameter) from first feeding at Whitebrook Fish Farm, Gwent, U.K. The fish were fed a standard diet during this period (Vextra, EWOS Ltd., U.K.) and were

graded regularly in order to ensure the fish were of approximately the same size. The trial started when the live weight of the individual fish was approximately 70g.

A random sample of thirty fish was removed from the population and the fish were killed using an overdose of the anaesthetic 2-phenoxyethanol (0.4ml per litre) followed by a blow to the head using a wooden priest (percussion stunning). The fish were weighed and measured to find the fork length (the distance between the tip of the nose and the fork of the tail). They were then eviscerated and reweighed. The viscera and the rest of each carcass were bagged separately and frozen for proximate analysis.

2.2.3 Husbandry

Twelve adjacent tanks of 1m diameter, each with a working capacity of 470 litres were prepared. All were supplied with spring water from the same source. To each of the tanks in turn, ten fish chosen at random from the remainder of the population were added. This was repeated until each tank contained thirty fish.

After allowing two days for acclimatisation after the transfer, six tanks were randomly allocated to the low oil diet and six to the high oil diet. The amount fed was calculated weekly thus:

The weight of the fish in each tank at start of week 1= x (derived from sample weight)

Assume doubling of weight over one month and linear growth, therefore

start of week 2 weight = $5x / 4$

start of week 3 weight = $6x / 4$

start of week 4 weight = $7x / 4$

From feed tables (EWOS for 26% oil trout feed), the optimum rate of feed should be *y*% of the weight of the fish at a given temperature. The fish were to be fed at 95% of *y*%, to ensure all of the food was eaten.

Therefore for the four weeks the feed to each tank was:

Week 1:	*x* x *y*% x 95%
Week 2:	5*x* / 4 x *y*% x 95%
Week 3:	6*x* / 4 x *y*% x 95%
Week 4:	7*x* / 4 x *y*% x 95%

After four weeks of feeding, ten fish from each tank were randomly selected and killed using an overdose of the anaesthetic 2-phenoxyethanol (P1126, Sigma, UK) followed by a percussion stun. The fish were weighed and other measurements described below in section 2.2.4 were carried out. The weight of the fish from each tank was used to calculate the next four weeks' feeding regime. The removal of the ten fish from each tank allowed for the growth of the other fish; if they had not been removed the tanks would have become overcrowded.

Four weeks later a second sample of ten fish was removed from each tank as before. After another four weeks the final ten fish from each tank were removed and killed. They were weighed, measured, eviscerated and reweighed as before. Each carcass and set of viscera were then separately bagged and frozen for proximate analysis.

2.2.4 Whole Fish Measurements

After being killed, the fish were wiped with a dry piece of absorbent paper to remove surface water. They were then weighed to determine the "live weight", before being

eviscerated. This involved the removal of the stomach, liver, intestine and swim bladder. The kidneys, heart and gills were left in place.

Following evisceration the fish were reweighed on the same balance. The fork length of each fish was then determined. From the whole fish measurements the following were determined:

$$\text{Condition Factor, K} = \frac{\text{Live Weight (kg)} * 1000}{(\text{Fork Length (m)})^3}$$

The condition factor gives information about the conformation of the fish. The condition factor normally varies from 1.00 to 1.50 in farmed salmonids (pers. comm. R. McKinney, BOCM Pauls, Renfrew, U.K.). A long thin fish will have a low condition factor and a short fat fish will have a high condition factor.

$$\text{Dress Out \%} = \frac{\text{Eviscerated Weight}}{\text{Live Weight}} \times 100\%$$

The dress out percentage gives information about the loss of weight following evisceration. A high visceral weight will result in a low dress out percentage. This indicates a higher level of waste for the fish farmer. Dress out percentage normally varies between 80% and 90%.

The determination of the live weight, the condition factor and the dress out percentage at four weekly intervals following the random sampling from each tank allowed the effect of the diets on the growth and conformation of the fish to be monitored.

2.2.5 Proximal Analysis

The initial and final samples were subjected to proximal analysis of both carcass and viscera. The carcasses were thawed and filleted to remove the head, skin and backbone. The resulting fillets from each fish were homogenised, weighed and freeze-dried to a constant mass. The change in fillet weight gave the moisture content. The flesh was then milled by passing through a Cyclotech Mill (Perstorp Analytical, Bristol, U.K.) and the resulting millings from each fish mixed thoroughly. Sub-samples were then taken for ash, nitrogen, lipid and total energy determination. Similarly the entire visceral sample from each fish was homogenised, freeze dried, milled and samples taken for these analyses.

The ash content was determined by placing a 2g freeze-dried sample in a furnace at 600˚C for four hours. The sample was then desiccated and reweighed. The ratio of the remainder (the ash) to the dried sample before heating gave the percentage ash for the dry matter. The percentage ash of the wet weight could then be calculated for each individual fish.

Total nitrogen content of a dried sample was measured using a Leco FP428 nitrogen analyser (Leco Instruments, Stockport, Cheshire, U.K.). This was then converted to an estimate of total protein content by multiplying the total nitrogen by 6.25 (Holland *et al.*, 1991). The percentage protein of the wet weight was calculated for each individual.

Lipid was analysed using the soxhlet method. Freeze-dried samples were placed in an automatic soxhlet analyser and the lipid extracted using petroleum ether (Rathburns, Walkerburn, U.K.). The weight of lipid after evaporation of the solvent was determined, and hence the per cent lipid of the dry and wet weights determined.

Total energy was determined using an adiabatic bomb calorimeter (Gallenkamp). Freeze dried samples were individually placed in this and the total energy of each dried sample calculated and converted to the energy of the original wet sample.

2.2.6 Data Analyses

The results of the mean weighings and measurements from each tank were compared using a one factor analysis of variance (ANOVA) using StatView SE+ Graphics (Abacus Concepts Inc., Berkeley, U.S.A.). The values for the proximate analyses of the flesh and viscera samples were converted from percentages to absolute values for the whole fish. Graphs were plotted of both the proximate composition against the live weight and the logarithm of the proximate composition against the logarithm of the live weight, following Shearer (1994). As the proximate composition was found to be correlated to the live weight, analysis of covariance (ANCOVA) was carried out on the results of the proximate analyses, using live weight as the covariate (Genstat 5, release 3.1, Lawes Agricultural Trust, Rothamstead, U.K.). The analysis of covariance was also carried out on length and dressed weights, which were also closely correlated to live weight.

2.3 Results

2.3.1 Environmental Conditions

Throughout the trial the percentage oxygen saturation and the water temperature were monitored in all tanks (figures 2.3.1 and 2.3.2 respectively). Differences in oxygen saturation between the tanks were minimised by altering the amount of inflowing water — increasing it to raise the oxygen saturation and *vice versa*. The daily mean saturation ± standard error for all tanks throughout the trial was 77.0 ± 0.87%, which was above the level where fish start to show stress caused by low oxygen (Smart, 1981).

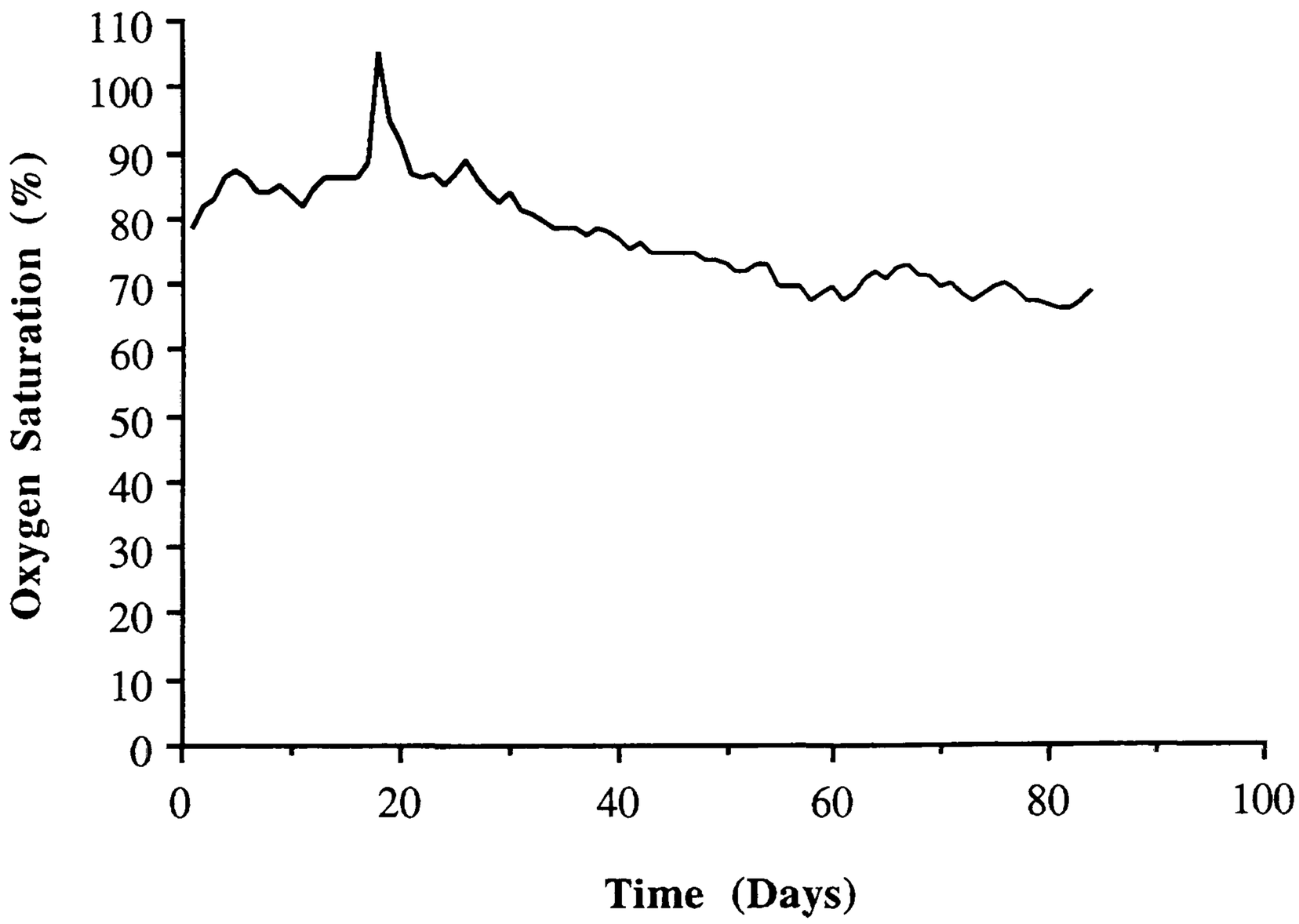

Figure 2.3.1: Percentage oxygen saturation of the water in the tanks during the trial.

The water temperature during the experiment was at ambient (figure 2.3.2). The temperature range was 11.2˚C to 19.5˚C, with the temperature dropping during the trial as autumn set in. The reduction in temperature helped to reduce the effects of any overcrowding problems in the tanks prior to sampling, when the weight of the

fish in the tanks was at its greatest. At lower temperatures the fish were less active and would have produced less waste, especially ammonia which is toxic to the fish and causes a reduction in appetite even at low levels (Smart, 1981).

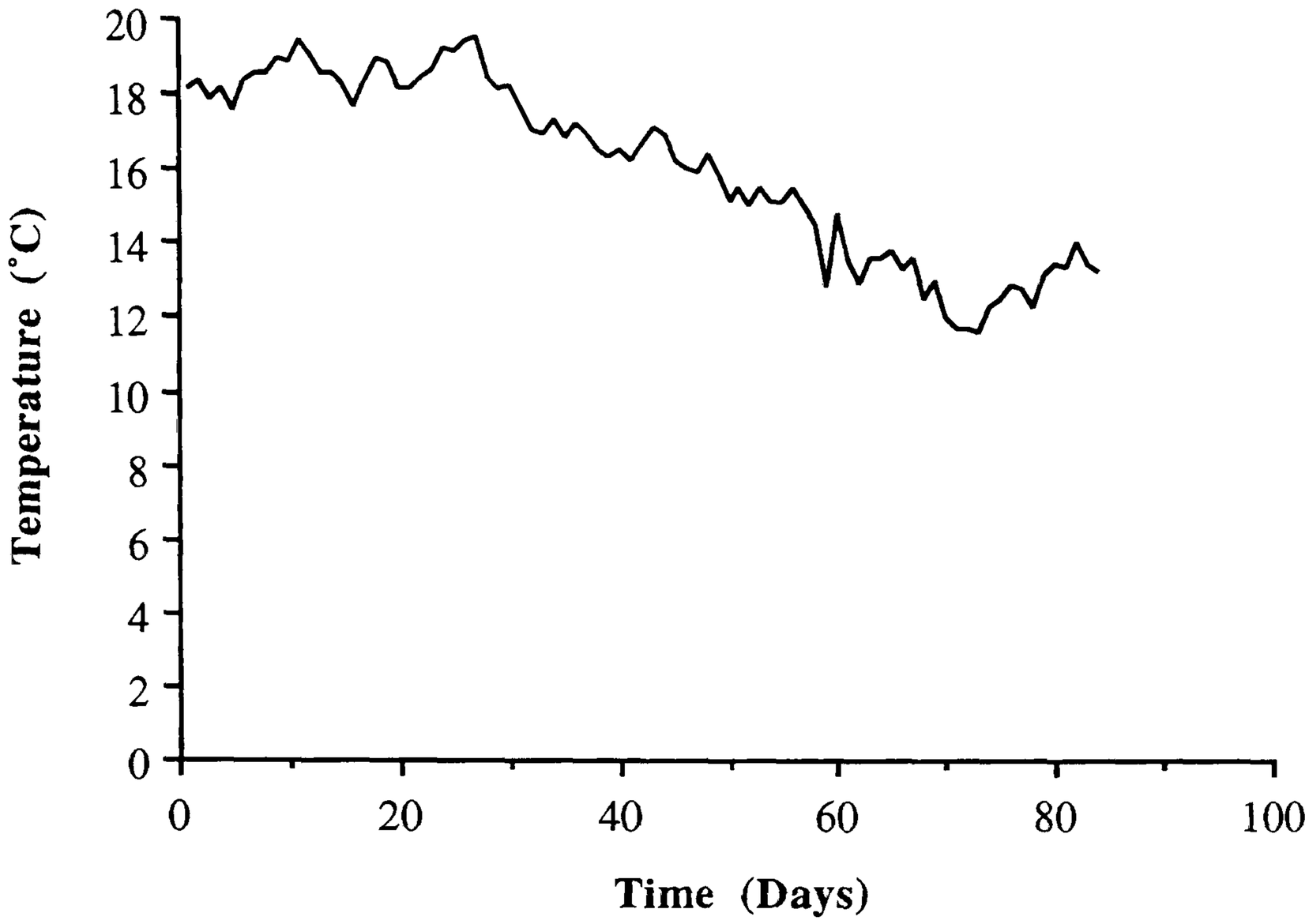

Figure 2.3.2: Mean water temperature in the tanks during the trial.

2.3.2 Initial Measurements

Table 2.3.1 gives the results of the measurements carried out on the initial sample of fish, which are a reference point for the rest of the trial.

Table 2.3.1: Initial measurements on the whole fish (n=30).

Measurement	Mean	Standard Deviation	Standard Error
Live Weight (g)	75.7	12.32	2.25
Fork Length (cm)	19.6	1.03	0.19
Dressed Weight (g)	68.7	11.14	2.03
Dress Out (%)	90.8	1.31	0.24
Condition Factor, K	1.00	0.075	0.014

The feeding trial lasted for twelve weeks, with three further sample points every four weeks. At all the sample points, ten fish from each tank were weighed and measured, allowing their growth to be monitored.

2.3.3 Live Weight

The mean weight of the fish from each tank at each of the sample points is shown in table 2.3.2. The fish in all the tanks grew well during the trial, finishing at approximately five times the mean weight at the start of the trial. However, it can also be seen that the fish in tank 2 of the group fed the high oil diet did not grow as fast as the other fish in the final month of the trial. The reason for this is unknown, but may have been due to a low level of an unknown stress resulting in the fish losing appetite.

Table 2.3.2: Mean monthly weights from each tank (n=10). Standard errors are in parentheses.

Diet	Tank	Live Weight (g)		
		August	September	October
High Oil	1	127.5 (7.44)	235.2 (18.23)	378.3 (29.16)
High Oil	2	135.8 (9.95)	231.9 (10.73)	291.3 (27.27)
High Oil	3	142.6 (10.85)	281.8 (20.20)	357.6 (24.66)
High Oil	4	150.0 (10.40)	226.8 (12.90)	380.4 (29.64)
High Oil	5	136.6 (13.49)	203.9 (15.41)	389.3 (29.05)
High Oil	6	144.8 (8.23)	220.2 (18.36)	349.6 (25.06)
Low Oil	1	137.1 (8.20)	248.6 (22.42)	311.9 (24.94)
Low Oil	2	141.8 (12.93)	216.1 (9.14)	301.9 (28.59)
Low Oil	3	151.2 (6.54)	224.7 (12.05)	330.7 (24.93)
Low Oil	4	138.6 (7.64)	235.0 (13.36)	310.3 (27.14)
Low Oil	5	122.2 (9.78)	239.3 (15.30)	327.5 (26.23)
Low Oil	6	107.5 (7.05)	240.5 (12.66)	332.4 (40.63)

Figure 2.3.3 shows the overall mean weight for each dietary group. For the purposes of growth measurements, the individual fish are not statistically independent as there

are interactions between the fish within each tank, such as the formation of feeding hierarchies. However, the mean values from each tank are independent, and so in this trial $n = 6$. The growth of the low oil diet and high oil diet fed groups was similar for the first eight weeks and then the fish fed the high oil diet increased their growth rate over the last four weeks. This resulted in heavier fish fed the high oil diet, with a mean weight and standard error of 358 ± 14.6g compared with 319 ± 5.2g. The results of a one factor ANOVA on the final-weight data showed that the difference was significant ($p<0.05$).

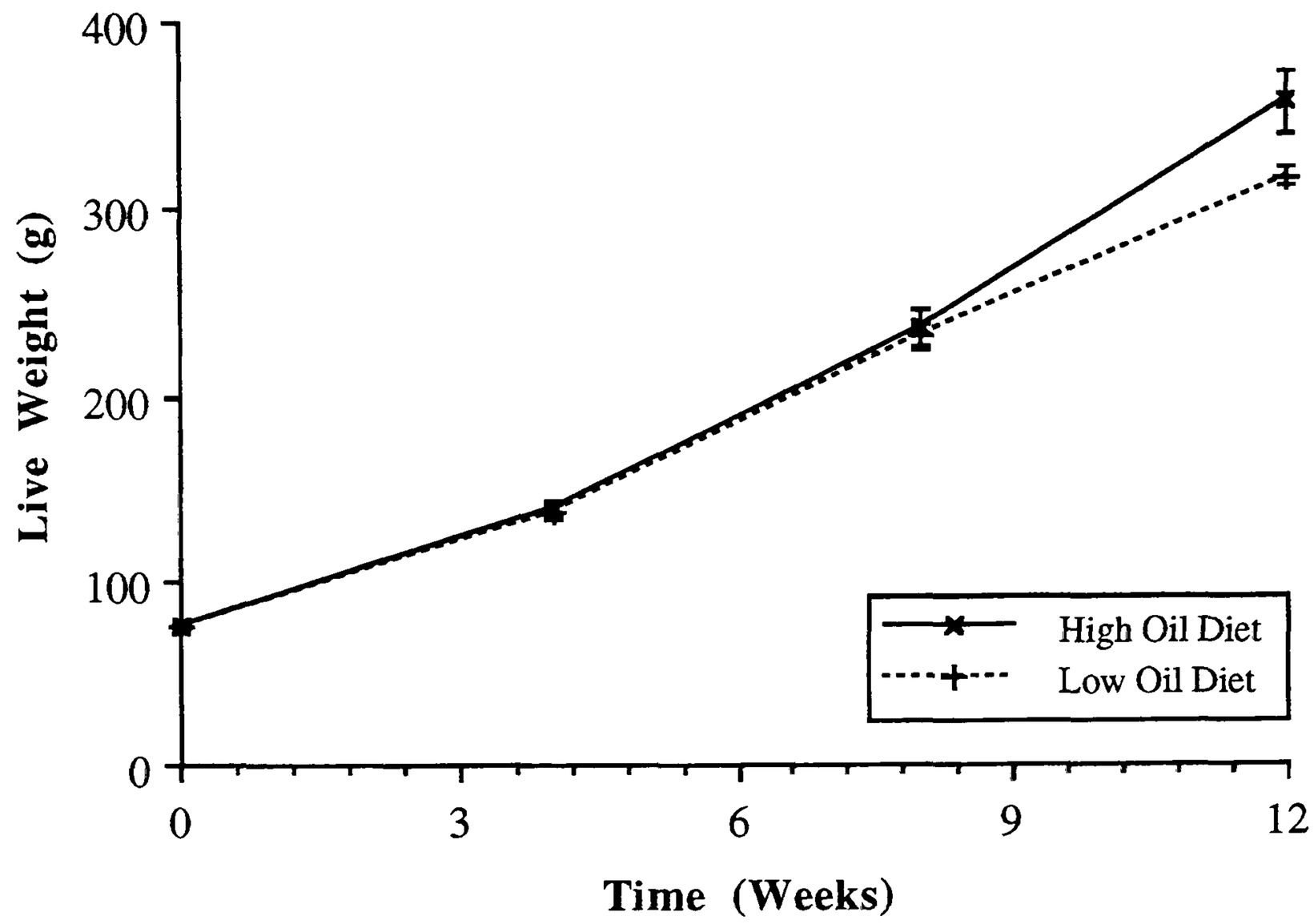

Figure 2.3.3: Mean live weight of fish fed the two diets (± s.e.m.). (n= 6 tanks)

2.3.4 Length

The fork length of the fish increased during the trial in all tanks (table 2.3.3). However, at the end of the trial there was no significant difference in the mean length of the fish fed the two diets (28.7 ± 0.35cm for the high oil diet fed fish compared with 28.3 ± 0.22cm for the fish fed the low oil diet). The results of a one factor ANOVA confirmed this ($p>0.05$).

Table 2.3.3: Mean length of the fish from each tank during the trial (n=10 fish). Standard errors are in parentheses.

Diet	Tank	August	September	October
High Oil	1	21.5 (0.48)	25.9 (0.69)	28.6 (0.53)
High Oil	2	22.5 (0.52)	25.5 (0.44)	27.2 (0.90)
High Oil	3	22.4 (0.67)	26.9 (0.51)	29.1 (0.58)
High Oil	4	22.7 (0.47)	25.5 (0.35)	29.5 (0.51)
High Oil	5	21.8 (0.74)	24.5 (0.53)	29.3 (0.51)
High Oil	6	22.1 (0.45)	25.9 (0.50)	28.7 (0.58)
Low Oil	1	21.9 (0.49)	26.4 (0.75)	27.8 (1.01)
Low Oil	2	21.8 (0.71)	25.6 (0.34)	27.9 (0.75)
Low Oil	3	22.8 (0.33)	25.5 (0.53)	28.7 (0.64)
Low Oil	4	22.2 (0.40)	26.0 (0.46)	27.8 (0.82)
Low Oil	5	21.2 (0.59)	25.7 (0.57)	28.6 (0.53)
Low Oil	6	20.8 (0.37)	25.9 (0.44)	29.1 (1.14)

The length of each fish was plotted against its weight at the end of the trial (figure 2.3.4). Regression analysis of all the data (Genstat 5) showed the importance of the live weight to the length, accounting for 83.6% of the variance. This indicated that analysis of covariance would be appropriate for determining the effect of the diets on the length of the fish.

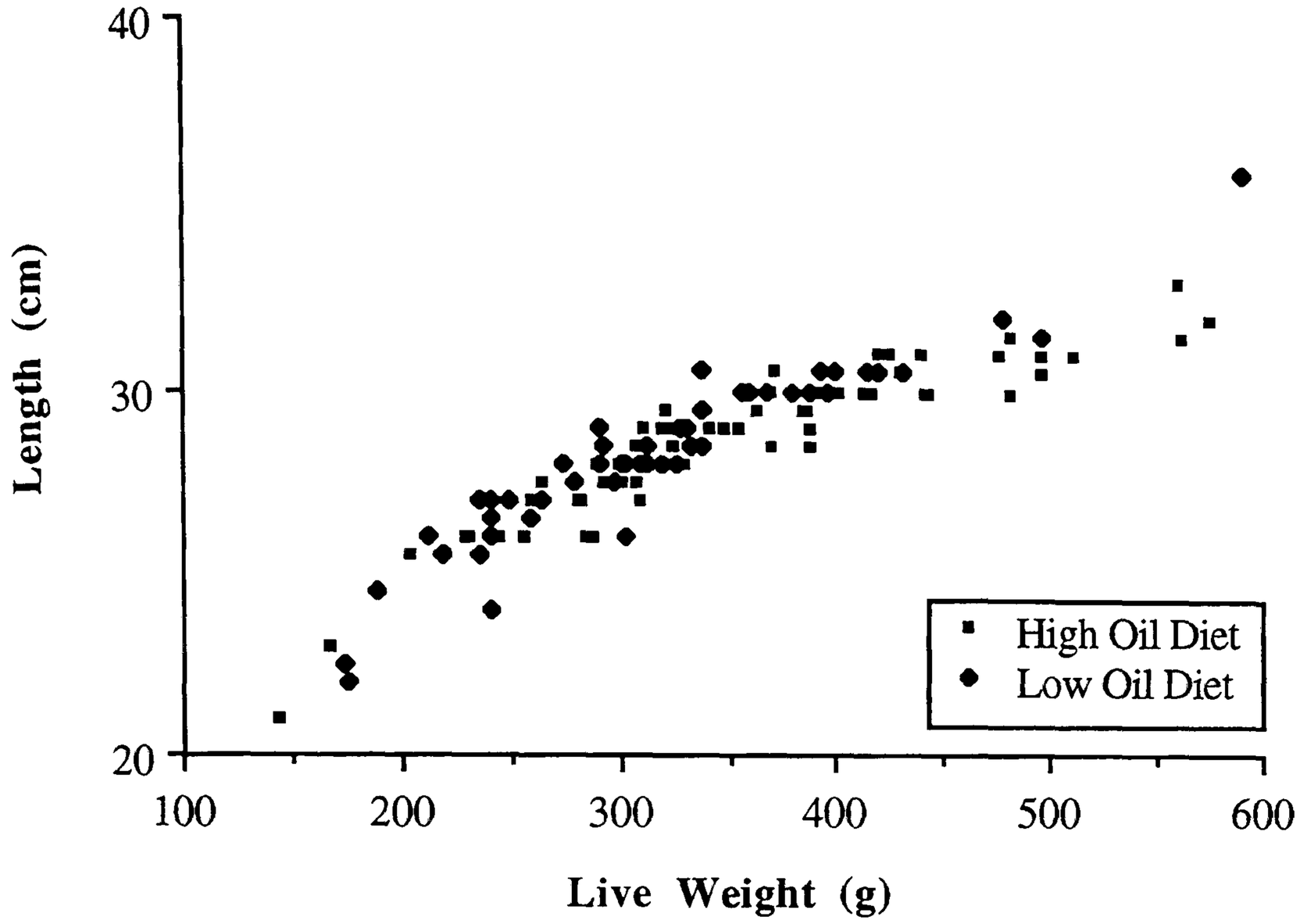

Figure 2.3.4 Fork length of the fish plotted against the live weight.

	d.f.	variance ratio
Regression	1	587

2.3.5 Condition Factor

The condition factor of the fish was determined from the length and live weight of each fish. As described above, it gives information on the conformation of the fish: a higher condition factor indicates a heavier fish for a given length.

Table 2.3.4 shows the mean condition factor for each tank at each of the sample points. During the second month of the trial, fish in tank 6 of the high oil diet and tank 2 of the low oil diet lost condition and similarly in the final month the fish in tank 6 of the low oil treatment group lost condition. The reason for this is not known, as the fish in these tanks had all increased in weight during the trial. The effect was probably due to an unknown stress in these tanks causing the fish to become thinner.

Table 2.3.4: Mean condition factor of the fish from each tank during the trial (n=10 fish). Standard errors are in parentheses.

Diet	Tank	August	September	October
High Oil	1	1.27 (0.022)	1.33 (0.030)	1.58 (0.048)
High Oil	2	1.18 (0.024)	1.40 (0.046)	1.41 (0.042)
High Oil	3	1.26 (0.041)	1.43 (0.055)	1.43 (0.031)
High Oil	4	1.27 (0.026)	1.36 (0.034)	1.45 (0.050)
High Oil	5	1.27 (0.037)	1.38 (0.084)	1.51 (0.035)
High Oil	6	1.34 (0.038)	1.27 (0.091)	1.45 (0.027)
Low Oil	1	1.29 (0.035)	1.33 (0.068)	1.45 (0.027)
Low Oil	2	1.35 (0.069)	1.28 (0.019)	1.38 (0.068)
Low Oil	3	1.26 (0.023)	1.35 (0.043)	1.38 (0.031)
Low Oil	4	1.26 (0.036)	1.32 (0.037)	1.41 (0.019)
Low Oil	5	1.25 (0.049)	1.40 (0.030)	1.38 (0.037)
Low Oil	6	1.18 (0.026)	1.37 (0.053)	1.32 (0.029)

Figure 2.3.5 shows how the mean condition factor of the fish fed the two diets changed during the trial. The figure shows the mean of the mean condition factors from the six tanks fed each of the diets.

At the start of the trial the condition factor of the fish was very low due to the small rations the fish had received prior to the trial (table 2.3.1). After four weeks of the trial diets the condition factor of the fish in both dietary treatments had risen dramatically, but there was no difference between the treatments. From this point, the rate of increase of condition factor of the fish fed the low oil diet slowed, but the fish fed the high oil diet continued to increase in condition factor (figure 2.3.5). By the final sample point the condition factor of the fish fed the low oil diet was 1.38 ± 0.017 compared to the fish fed the high oil diet at 1.47 ± 0.026. This was shown to be a significant difference by a one factor ANOVA ($p<0.05$).

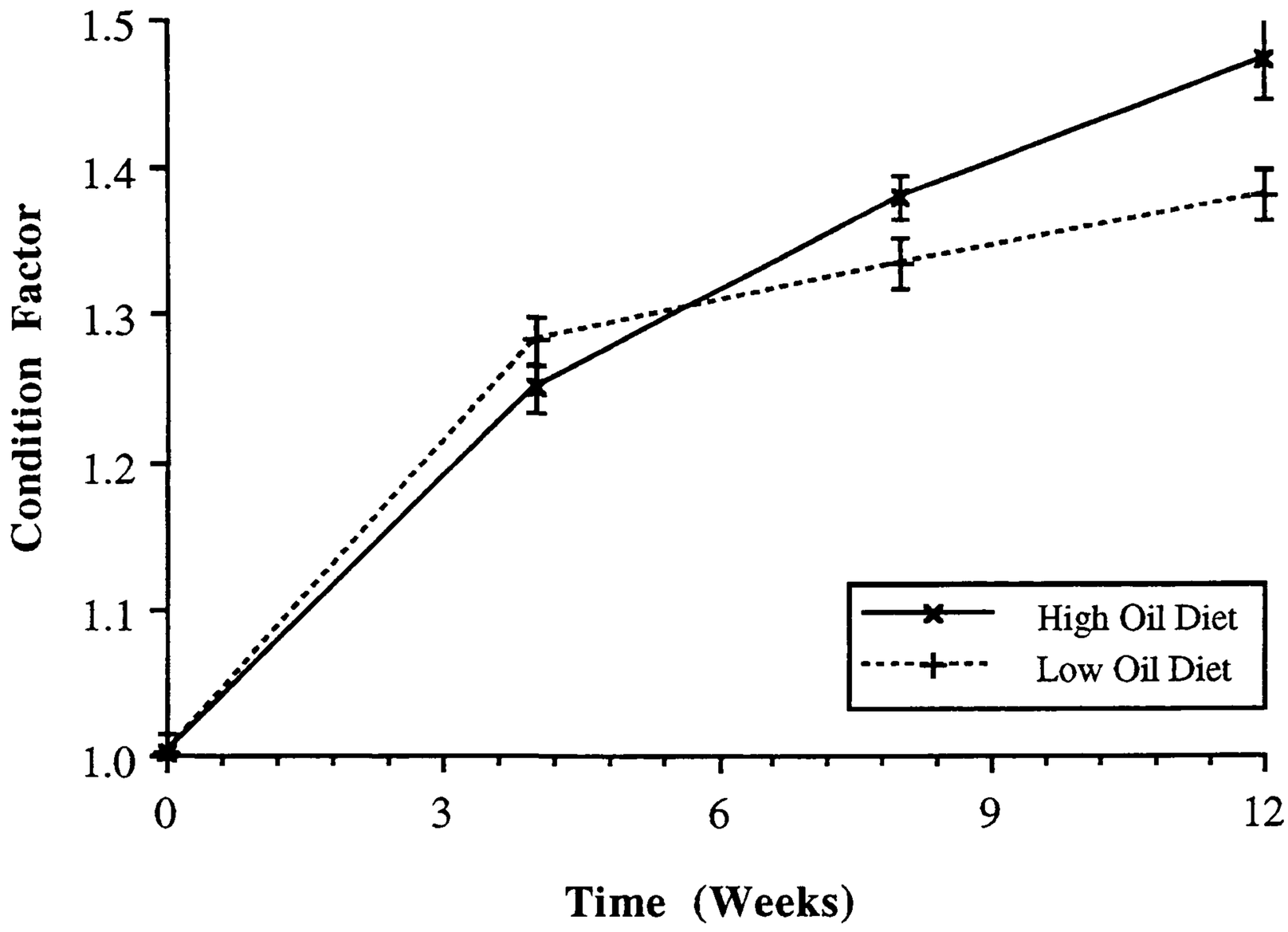

Figure 2.3.5: Mean condition factor of the fish throughout the trial (± s.e.m.). *n*= 6 tanks.

As the condition factor was calculated from the live weight there was no reason to look for a relation between the two variables.

2.3.6 Dressed Weight

The dressed weight of the fish increased as the fish grew in all tanks (table 2.3.5). It can be seen however that the fish in tank 2 (high oil diet) did not grow as much as the others in the last month. The reason for this, as stated above (section 2.3.3), is unknown.

Table 2.3.5: Mean dressed weight of the fish throughout the trial. Standard errors are in parentheses (n=10).

Diet	Tank	August	September	October
High Oil	1	113.0 (6.94)	197.0 (14.79)	329.6 (24.42)
High Oil	2	120.3 (8.72)	194.4 (9.65)	253.8 (23.07)
High Oil	3	126.0 (9.60)	238.6 (16.98)	313.2 (20.32)
High Oil	4	131.7 (9.19)	194.3 (10.65)	333.7 (26.37)
High Oil	5	121.8 (12.32)	176.3 (13.33)	342.4 (26.40)
High Oil	6	128.1 (7.33)	189.0 (15.91)	307.9 (22.44)
Low Oil	1	123.8 (7.40)	214.6 (20.09)	278.8 (21.94)
Low Oil	2	125.2 (10.75)	185.2 (7.55)	268.3 (24.90)
Low Oil	3	135.5 (29.5)	193.7 (10.47)	294.7 (22.20)
Low Oil	4	123.8 (6.81)	202.4 (11.04)	276.5 (24.66)
Low Oil	5	107.8 (8.24)	205.9 (13.46)	292.2 (23.74)
Low Oil	6	96.2 (6.58)	206.8 (10.31)	295.7 (35.61)

Figure 2.3.6 shows how the two diets affected the dressed weight. The effect is very similar to that on live weight, with a non-significant trend towards a greater dressed weight in the fish fed the high oil diet at the end of the trial (p=0.062).

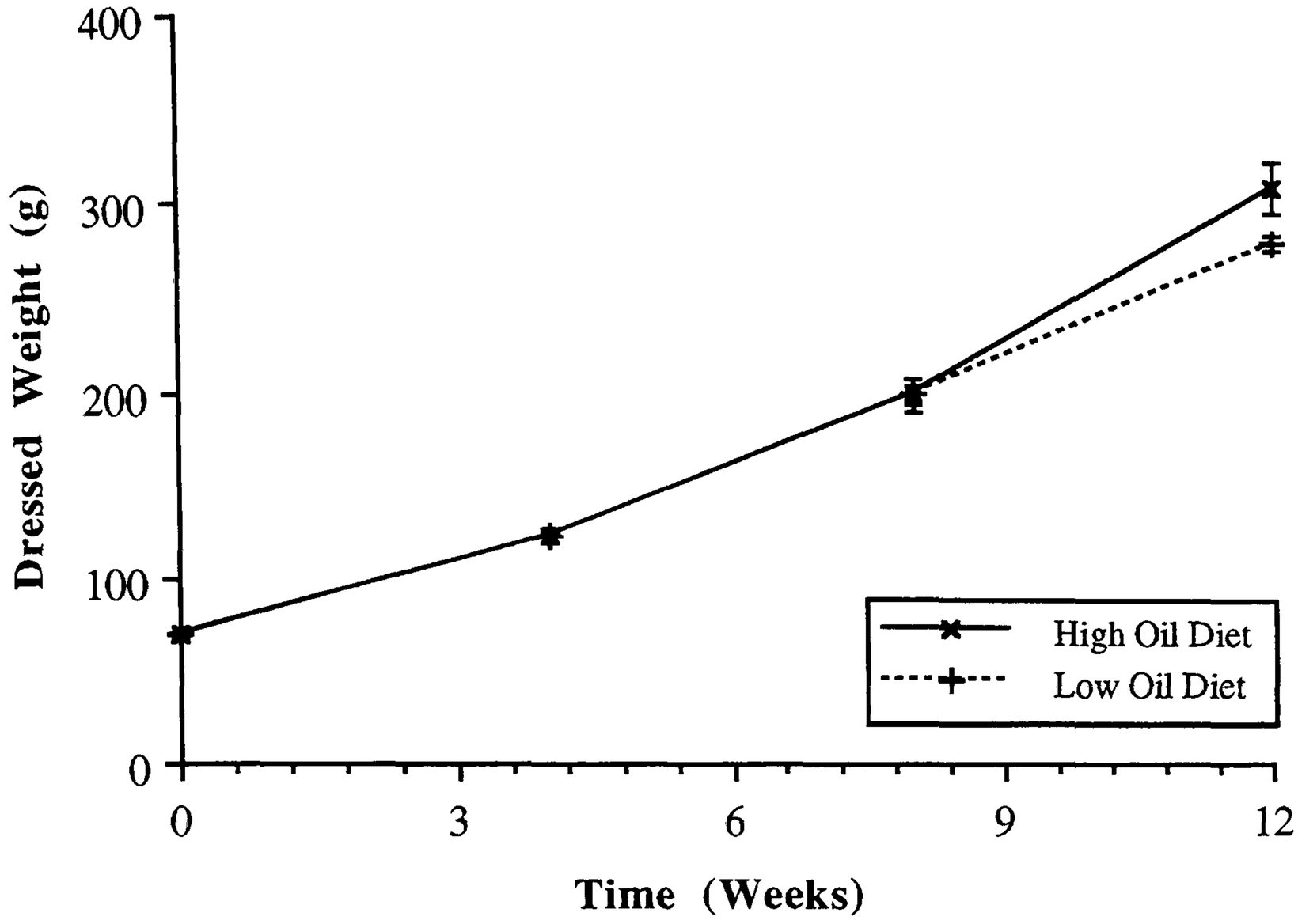

Figure 2.3.6: Mean dressed weight of the fish during the trial (± s.e.m.). n=6 tanks.

The plot of dressed weight against live weight showed that there is a very strong relationship between the two (figure 2.3.7). Regression analysis of all the data showed that the live weight accounted for 99.6% of the variance in the dressed weight, indicating that to determine the effect of the two diets on the dressed weight analysis of covariance would have to be used, with the live weight as the covariate.

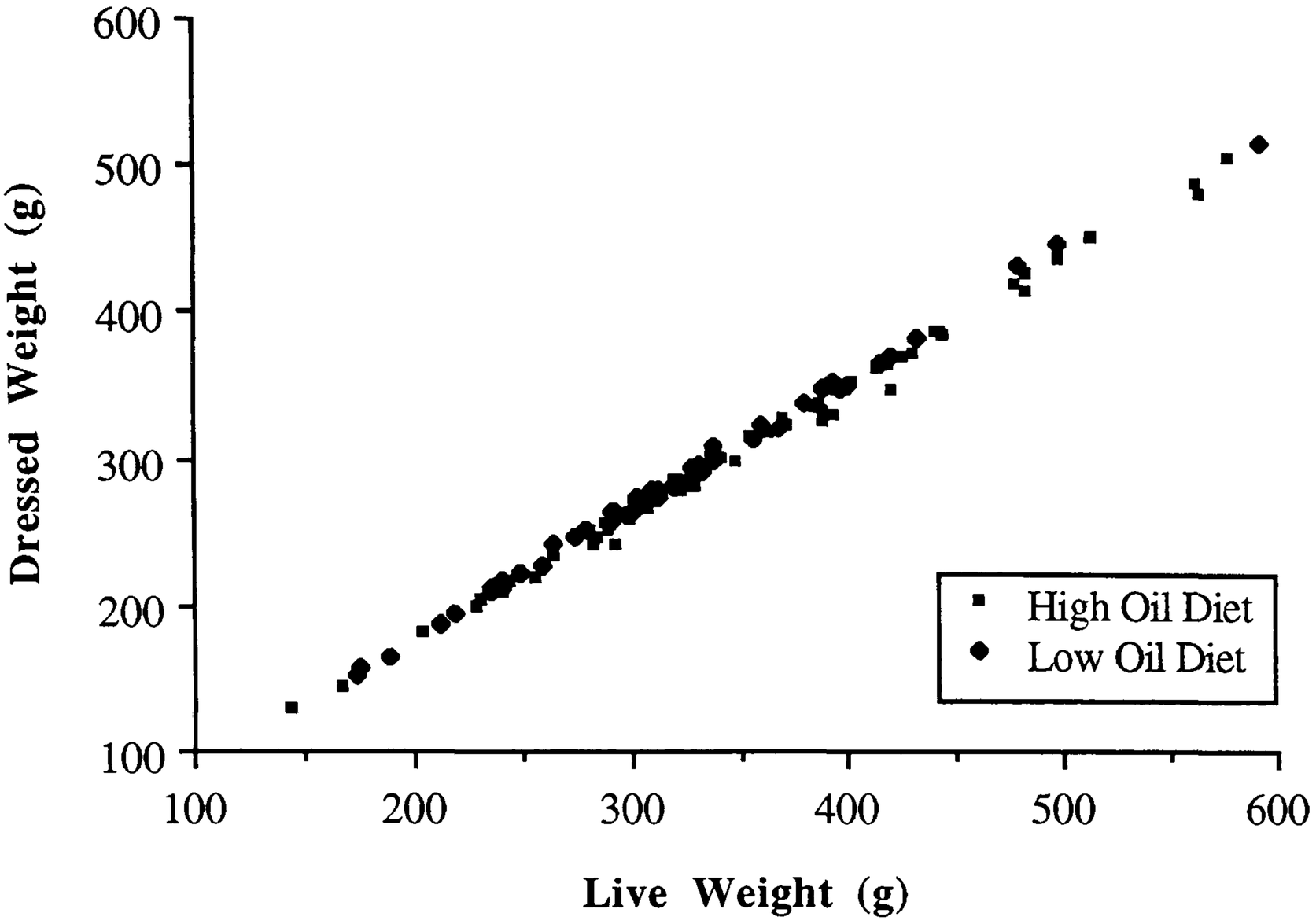

Figure 2.3.7 Dressed weight plotted against the live weight.

	d.f.	variance ratio
Regression	1	26600

2.3.7 Dress Out Percentage

The dress out percentage of the trout was initially high (table 2.3.1), owing to the small rations the fish had received prior to the trial, which would have decreased the size of the lipid reserves in the viscera. After four weeks of feeding the trial diets the dress out percentage had decreased for both dietary treatments (figure 2.3.8). At eight weeks there was a large decrease in dress out percentage in both dietary groups, before there was a rise at twelve weeks. The reason for this large decrease is not clear.

Figure 2.3.8 shows the mean of the mean dress out percentages for each of the treatments. This is not strictly a statistically legitimate procedure, but it helps to illustrate the changes in dress out percentage. A significant difference between the two dietary groups could be seen after four weeks ($p<0.05$) and remained throughout the trial, becoming highly significant at the end ($p<0.001$).

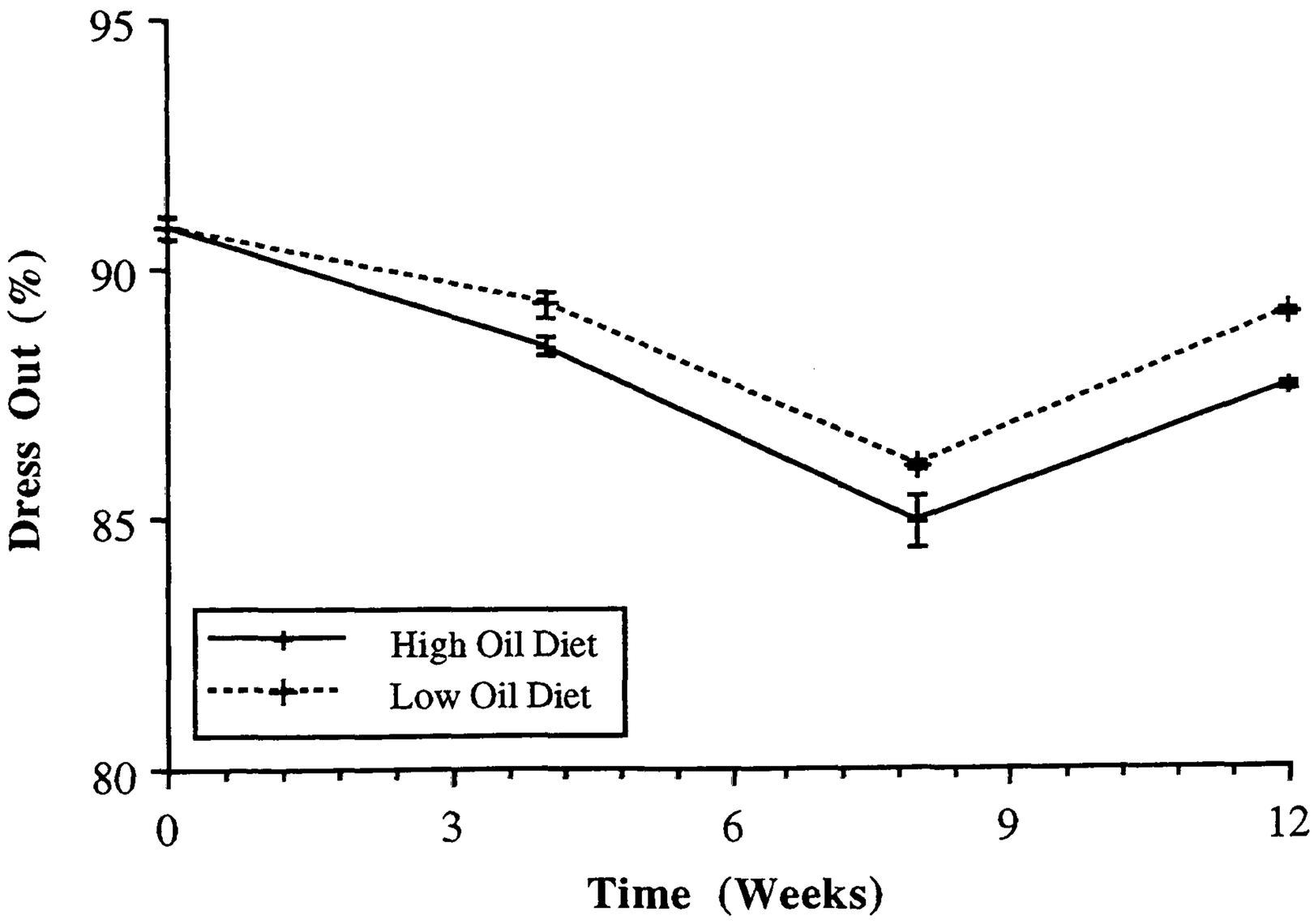

Figure 2.3.8: Mean dress out percentage of the fish during the trial (± s.e.m.). n= 6 tanks.

2.3.8 Fillet Analyses

The total fillet dry matter, ash, protein, lipid and energy contents were plotted against the live weight of the fish at the end of the trial (figures 2.3.9 to 2.3.13 respectively). Regression analyses on the data from each plot was used to determine the strength of the relationship of each of the parameters with the live weight (table 2.3.6). When the relation was strong for each parameter, analysis of covariance was used, with live weight as the covariate, to determine the effect of the diets on the parameter.

Table 2.3.6 Regression analysis of the fillet proximate composition against live weight of the fish for all fish (n=116).

Parameter	d.f.	variance ratio	Variance accounted for (%)
Fillet Dry Matter	1	7180	98.4
Fillet Ash	1	1750	93.8
Fillet Protein	1	2190	95.0
Fillet Lipid	1	1110	90.6
Fillet Energy	1	3840	97.1

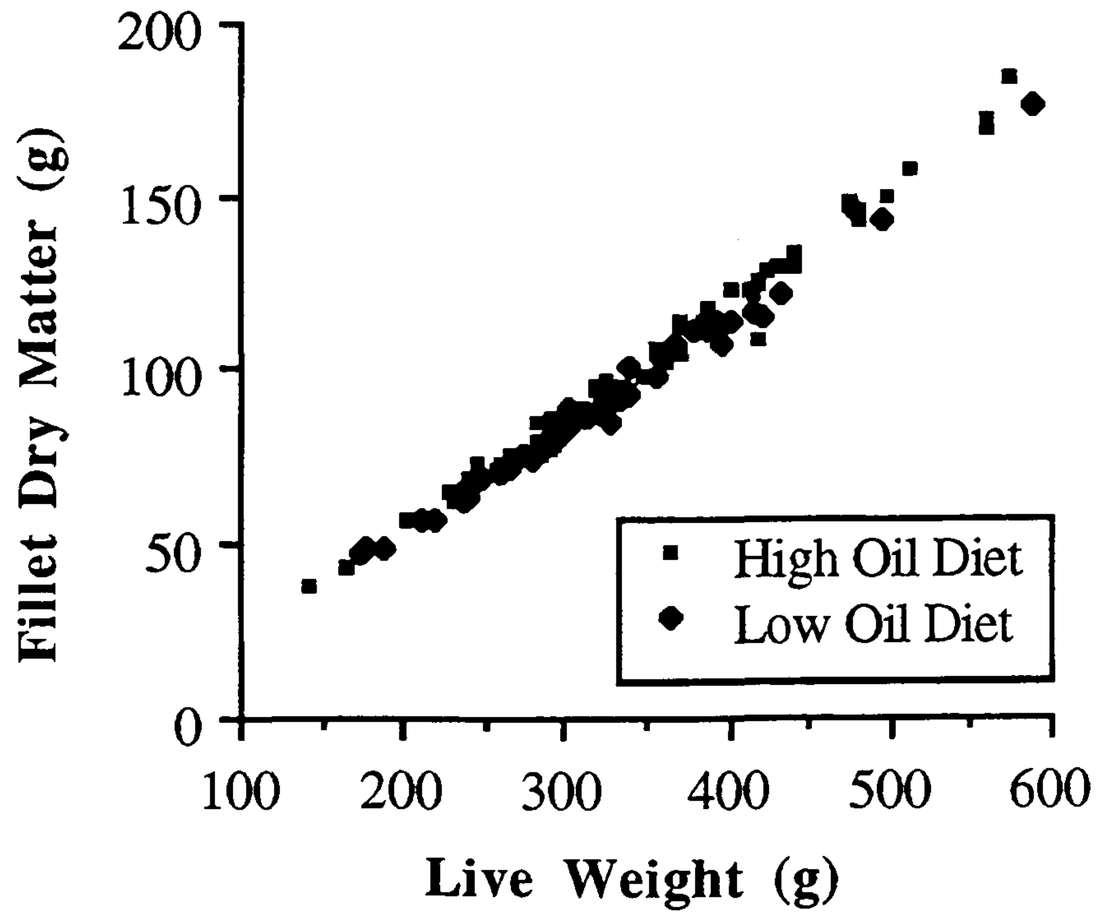

Figure 2.3.9 Fillet dry matter content plotted against live weight.

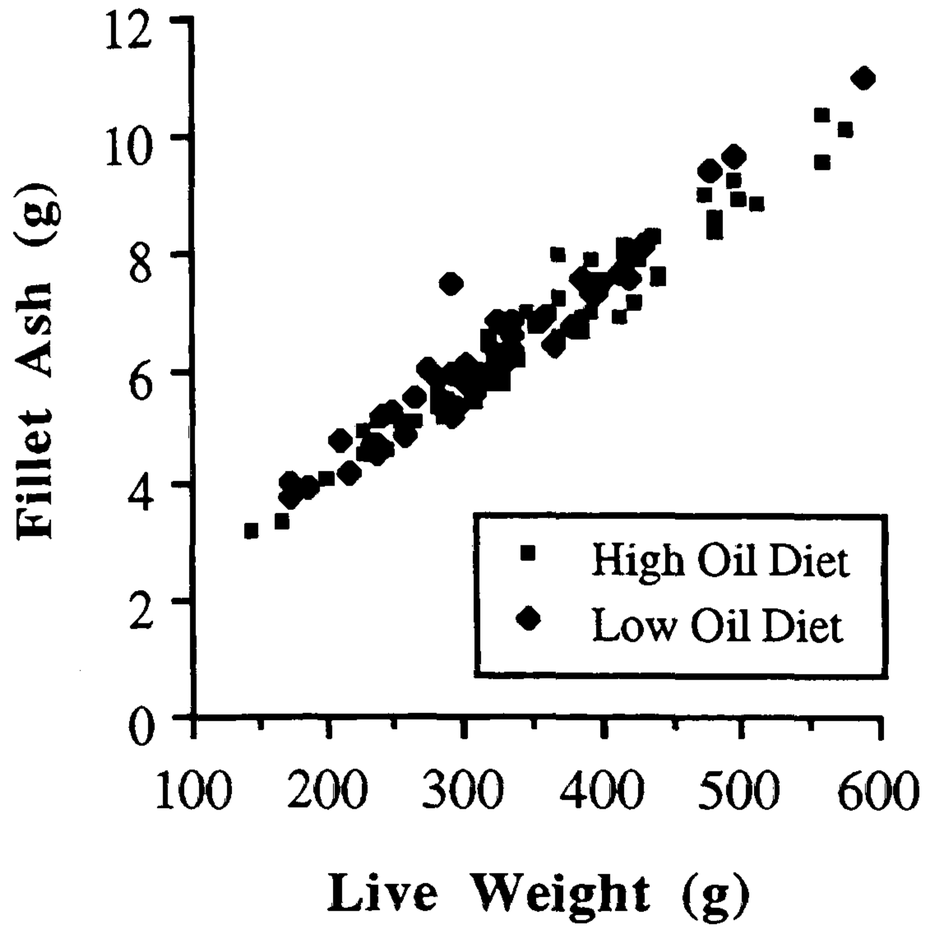

Figure 2.3.10 Fillet Ash

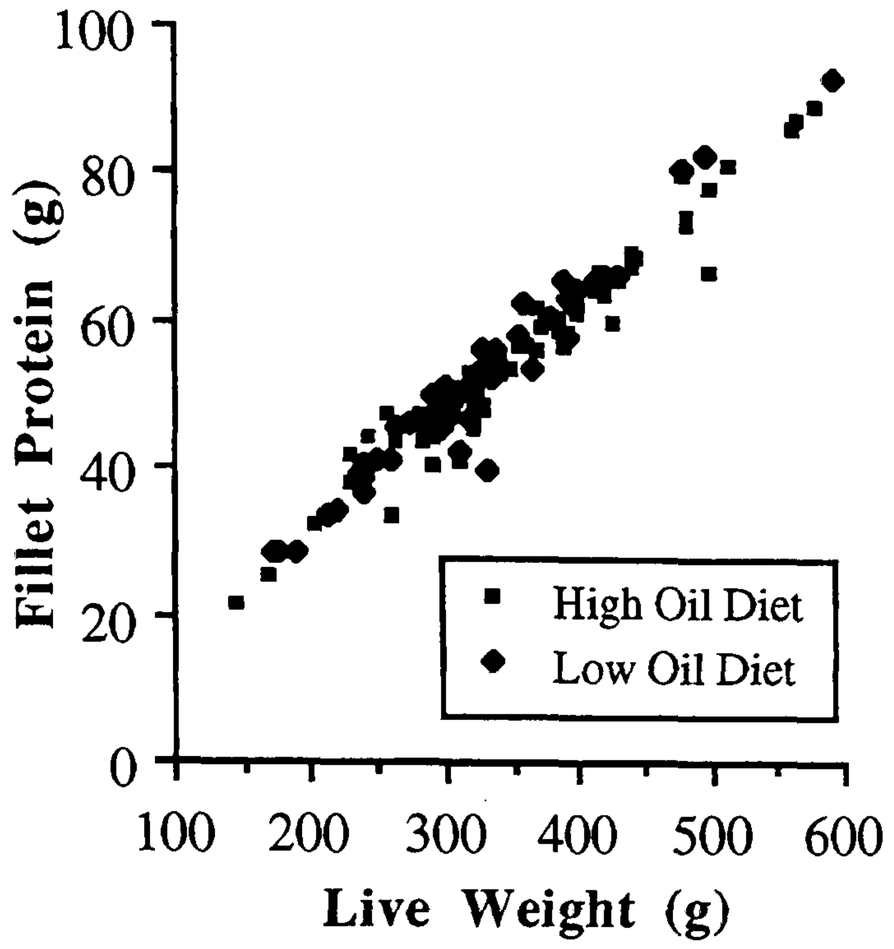

Figure 2.3.11 Fillet Protein

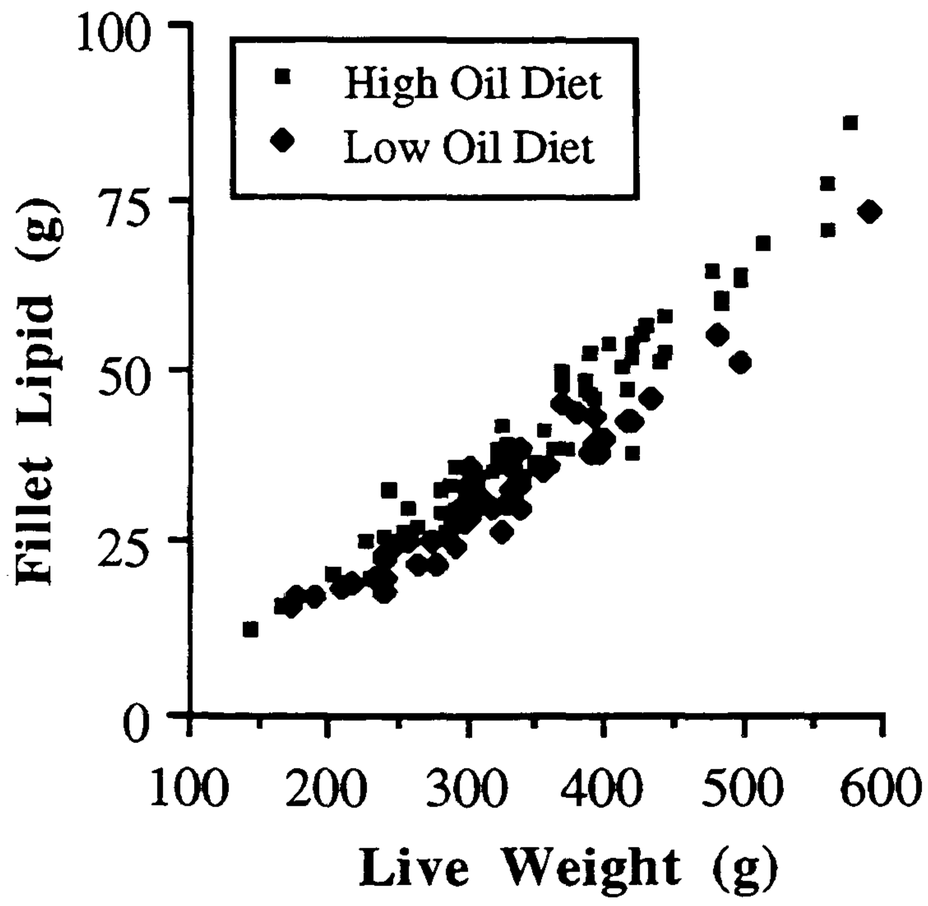

Figure 2.3.12 Fillet Lipid

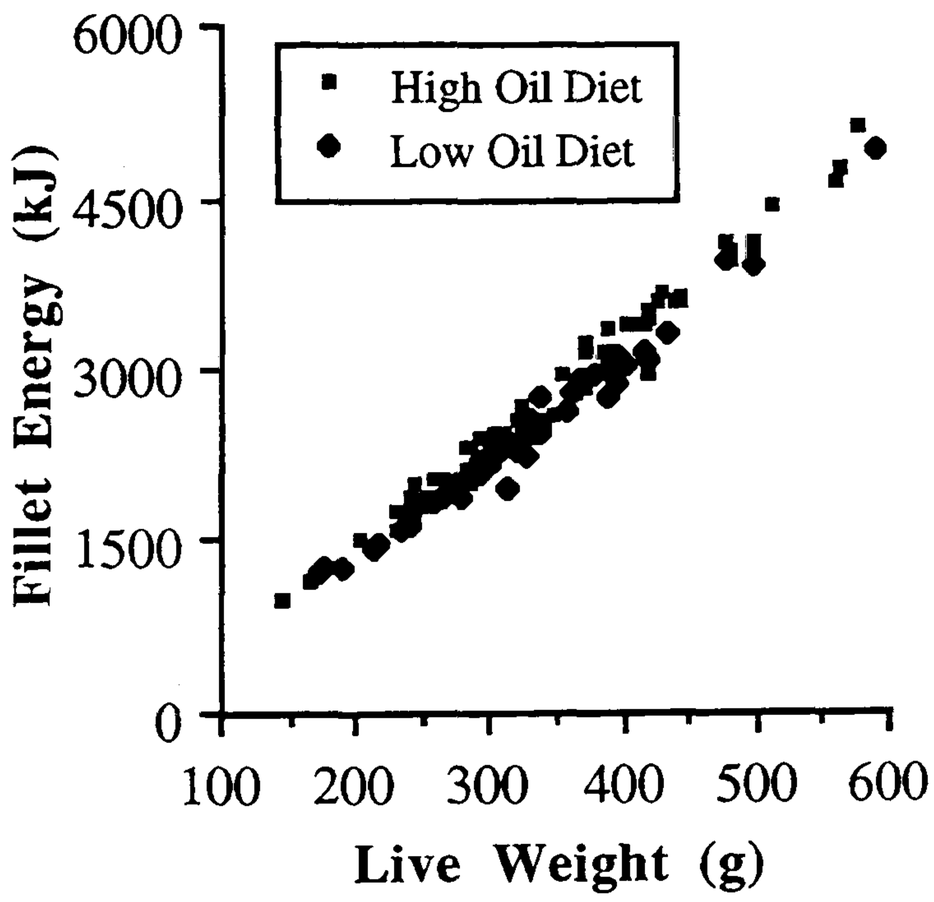

Figure 2.3.13 Fillet Energy

2.3.9 Visceral Analysis

The total visceral dry matter, ash, protein, lipid and energy contents were plotted against both the live weight and the visceral weight. The correlation for each was found to be greater against the visceral weight and so this is shown in figures 2.3.14 to 2.3.18 respectively. Regression analysis on all the data collected for each parameter showed the importance of the visceral weight and the percentage variance accounted for by the visceral weight (table 2.3.7). The visceral weight was then used as the covariate in the analysis of covariance in order to determine the effects of the diets.

Table 2.3.7 Regression analysis of the visceral proximate composition against visceral weight of the fish for all fish (n=116).

Parameter	d.f.	variance ratio	Variance accounted for (%)
Visceral Dry Matter	1	1840	94.1
Visceral Ash	1	98.7	46.4
Visceral Protein	1	81.5	41.6
Visceral Lipid	1	808	87.7
Visceral Energy	1	1130	90.9

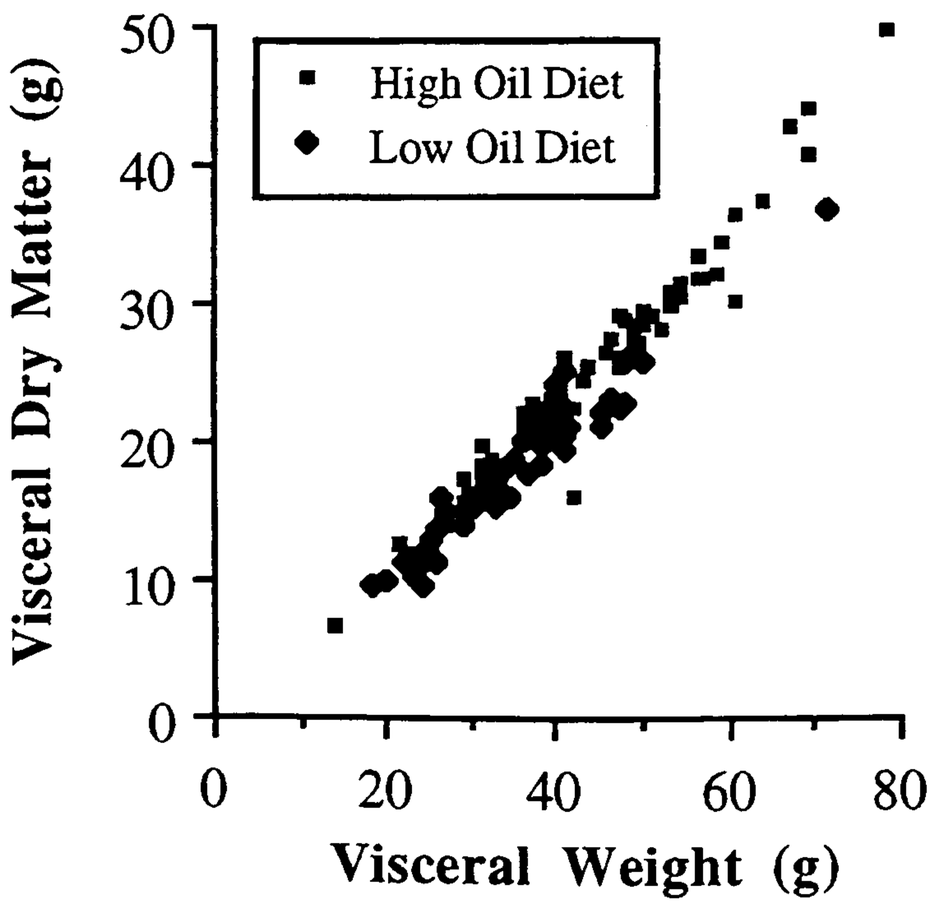

Figure 2.3.14 Visceral dry matter

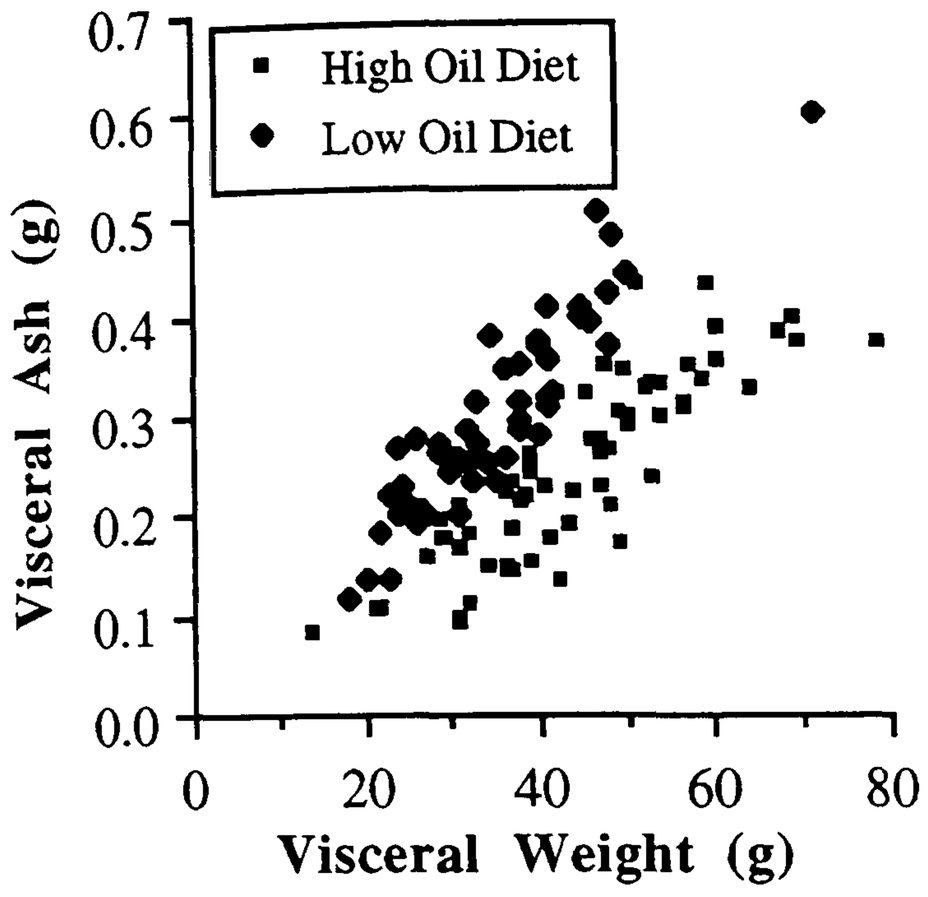

Figure 2.3.15 Visceral ash

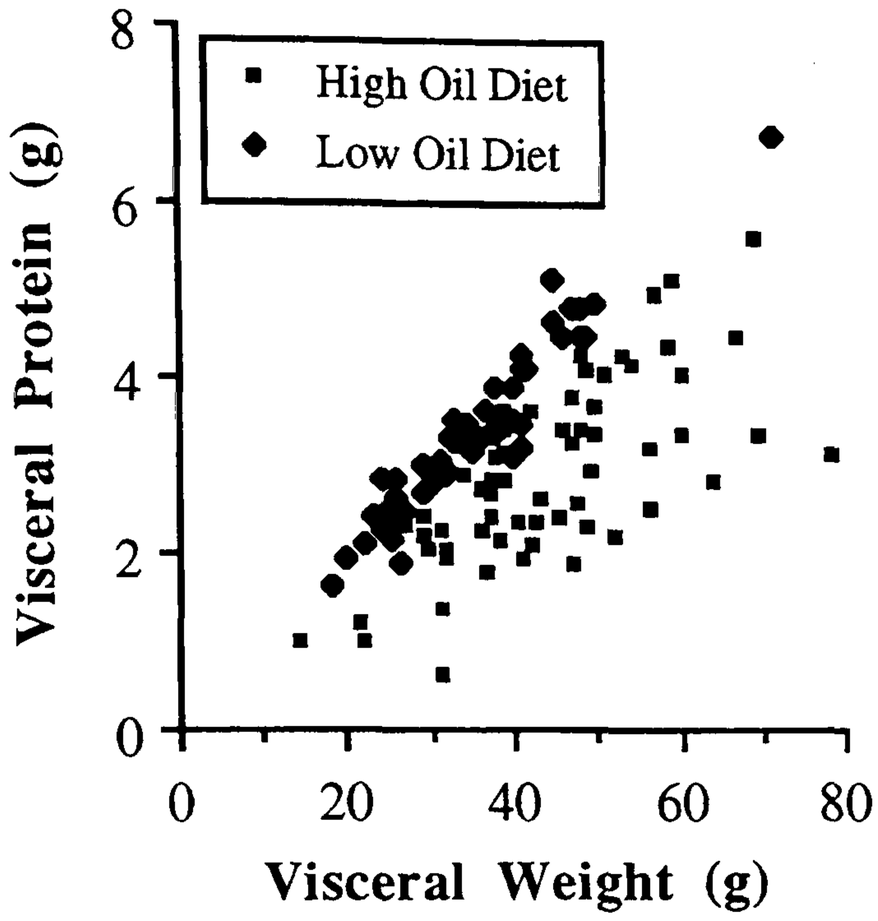

Figure 2.3.16 Visceral protein

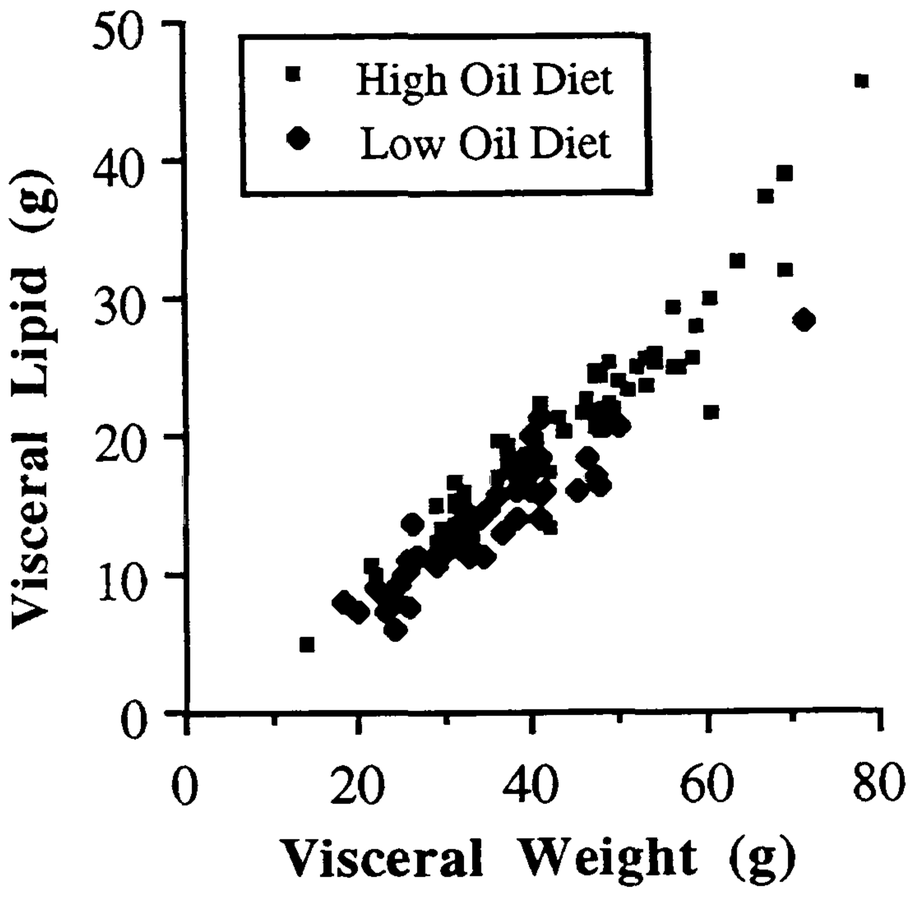

Figure 2.3.17 Visceral lipid

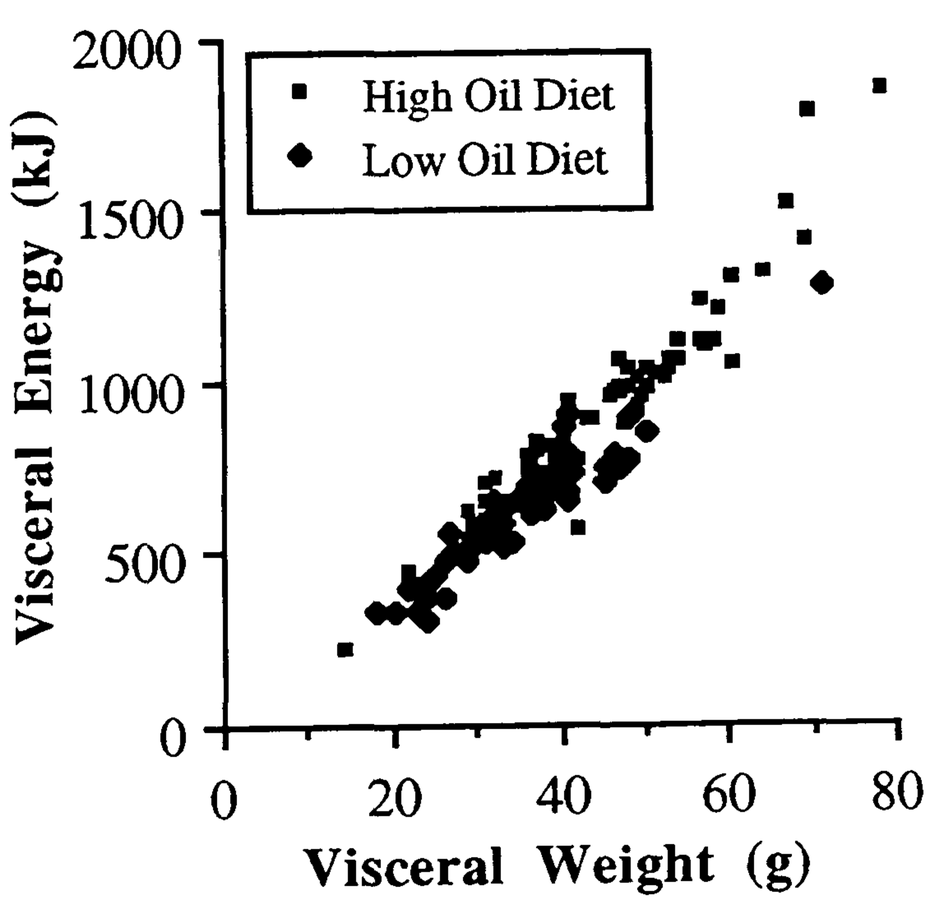

Figure 2.3.18 Visceral energy

2.3.10 Proximate Composition Relation to Weight

The relations between the live weight and the parameters determined by the proximate analyses were further investigated by plotting the logarithm of the parameter against the logarithm of the live weight and the visceral weight for the fillets and viscera respectively. A straight regression line was fitted to this data and the equation of the line determined (table 2.3.8). The slope of the line gives information on how the parameter changes with live weight (Shearer, 1994).

Table 2.3.8 Relation between logarithm of the parameters and the logarithm of live weight for the fillets and visceral weight for the viscera.

	Fillets		Viscera	
	Equation (y=)	r^2	Equation (y=)	r^2
Dry Matter	-0.83 + 1.11x	0.987	-0.42 + 1.10x	0.942
Ash	-1.30 + 0.83x	0.939	-1.94 + 0.85x	0.478
Protein	-0.72 + 0.97x	0.946	-0.72 + 0.75x	0.423
Lipid	-1.79 + 1.33x	0.912	-0.65 + 1.18x	0.879
Energy	0.51 + 1.17x	0.969	1.06 + 1.14x	0.915

2.3.11 Analysis of Covariance

Shearer (1994) indicated that live weight has great effects on other body parameters and the proximate composition of the carcass. The diets have been shown to affect the live weight of the fish (section 2.3.3) and the length, dressed weight and proximate composition of the fillets is correlated with the live weight (sections 2.3.4, 2.3.6 and 2.3.8 respectively). Analysis of covariance (ANCOVA) was therefore used to further analyse the results, with live weight as the covariate. For the visceral composition, which is correlated with visceral weight (section 2.3.9) the visceral weight was used as the covariate.

In order to perform the ANCOVA, the values were converted to those of a fish of standard live weight. This weight was the overall mean live weight of all of the fish at the end of the experiment, which was 340g. Using this weight, the effects of the diets on the measured parameters could be determined. The results of the analyses of the length, dressed weight and fillet composition are shown in table 2.3.9.

Table 2.3.9: Analysis of covariance of the results of the measurements and proximate composition determination, using live weight as the covariate. All results were corrected to a live weight of 340g.

Parameter	High Oil	Low Oil	df (Diet / Residual)	Variance Ratio	Signif.
Length (cm)	28.3	28.8	1 / 113	6.85	$p < 0.05$
Dressed Weight (g)	297.8	302.7	1 / 113	30.92	$p < 0.001$
Fillet Dry Matter (g)	98.98	96.03	1 / 113	20.91	$p < 0.001$
Fillet Ash (g)	6.32	6.50	1 / 113	6.47	$p < 0.05$
Fillet Protein (g)	53.43	54.77	1 / 113	5.13	$p < 0.05$
Fillet Lipid (g)	39.91	34.94	1 / 113	51.43	$p < 0.001$
Fillet Energy (kJ / g)	2710	2560	1 / 113	41.03	$p < 0.001$

Taking live weight into consideration, it was found that length decreases significantly ($p<0.05$) with increasing oil in the diet. This differed from the results of the ANOVA and highlights the need for an ANCOVA which takes live weight into account. Dressed weight also decreases with increasing oil in the diet ($p<0.001$), which implies that the viscera of the fish fed the high oil diet are heavier as there is more loss at dress out. The fillet proximate composition is highly dependent on the diet. Fillet ash and protein content decrease with increased dietary oil ($p<0.05$), whilst fillet dry matter, lipid and energy increase ($p<0.001$).

The visceral composition was analysed using the visceral weight as the covariate (table 2.3.10). The ash and protein contents of the viscera decrease with increased oil in the diet ($p<0.001$), whilst the dry matter, lipid and energy content increase ($p<0.001$).

Table 2.3.10: Analysis of covariance on the results of the proximate composition of the viscera, using visceral weight as the covariate. Results were corrected to a visceral weight of 39.9g.

Parameter	High Oil	Low Oil	df (Diet / Residual)	Variance Ratio	Signif.
Dry Matter (g)	22.78	20.83	1 / 113	33.82	$p < 0.001$
Ash (g)	0.22	0.33	1 / 113	145.4	$p < 0.001$
Protein (g)	2.66	3.74	1 / 111	77.01	$p < 0.001$
Lipid (g)	19.10	16.46	1 / 111	36.80	$p < 0.001$
Energy (kJ / g)	819.0	727.5	1 / 111	34.32	$p < 0.001$

2.4 Discussion

In this trial, there was no significant difference in the live weights of the fish fed the two diets for the first eight weeks of the trial. A difference only occurred at the end of the twelfth week ($p<0.05$), when there was a sudden divergence in the live weights of the two groups. This was unexpected as it is well established that high oil diets result in increased growth rates (for examples see Shearer, 1994). There was no apparent reason for this result, which implies that the growth of the fish fed the high oil diet was being impeded by some unknown factor. However, all fish appeared healthy throughout the trial and there was no large difference between the mean weight of fish in different tanks fed the same diet.

The length of the fish correlates strongly with the live weight ($p<0.001$). The dressed weight of the fish fed the two diets was the same for the first eight weeks of the trial. Then, following the pattern shown by the live weight, the fish fed the high oil diet showed a significantly higher dressed weight at the end of the trial ($p<0.001$). The correlation between the dressed and live weights is very strong ($p<0.001$).

As was also reported by Shearer (1994), the proximate compositions of the fillets and viscera in this trial were significantly affected by the live weight of the fish at the end of the trial. Plotting individual composition against the live weight shows very strong correlations, although the visceral composition shows stronger correlation with the visceral weight.

From the slopes of the regression lines of the log-log plots for each diet (section 2.3.10), it can be seen that fillet ash content decreases with increasing live weight (slopes are less than one) and that fillet dry matter, lipid and energy content increase (slopes are greater than one). This supports the findings of Shearer (1994) in his re-analysis of previous data using this method of regression analysis.

The ratio of fillet protein content to live weight appears to remain almost constant, with a regression line slope for the log-log plot very close to 1 (section 2.3.10). Shearer *et al.* (1994) showed that above a size of 100g the percentage protein of whole Atlantic salmon remained constant with increasing body weight. However, in fish below this size, the authors found that the percentage protein increased with increasing body weight (the slope of the regression line was greater than 1).

All the rainbow trout from this trial were above 100g live weight at the end of the trial. However, working on fish below that size, the data of Reinitz (1983), reanalysed by Shearer (1994), showed an increase in protein with increasing live weight. It would, therefore, appear that in rainbow trout a similar transition from increasing percentage protein to a constant percentage occurs with growth. Further work is required to find at which weight this change occurs.

The fillet lipid content found in this trial was strongly related to the live weight of the fish, increasing more rapidly as the live weight rose. Similarly fillet energy content rose more rapidly as the live weight increased. Both of these results confirmed the findings of Shearer (1994) in his reanalysis of previous data.

In the current trial, visceral weight increased approximately linearly with increasing live weight, as was also shown by the control group in the work of Weatherley and Gill (1983) on rainbow trout. However, the visceral composition changed slightly with increasing visceral weight. The dry matter, ash, lipid and energy levels showed a slight increase, whilst protein levels dropped.

The scatter graphs of the data against live weight show that generally the proximate composition is strongly related to the live weight. Therefore an analysis which takes live weight into account is required to determine the effect of the diets on the proximate composition. As the diets clearly affect the live weight of the fish

(section 2.3.3), with the fish fed the high oil diets being significantly heavier ($p<0.05$), this must be allowed for. The simplest method of doing this is to use analysis of covariance. This compares the proximate composition of the fish after adjusting them all to the same live weight. Using such analysis, it could be seen that the fish fed the high oil diet are slightly shorter ($p<0.05$), despite receiving the same amount of protein in the diet as the fish fed the low oil diets. This could be because the fish require the protein for gains in weight as well as length. Therefore the protein may be partly restricting for the increase in length of these heavier fish.

The dressed weight of the fish fed the high oil diets is significantly lower, with a dress out percentage of 87.8% compared with 89.3% in the fish fed the low oil diets using the figures obtained from the ANCOVA (table 2.3.9). This increase is in part caused by the increase in lipid deposited in the viscera in the fish fed the high oil diets. The viscera appear to be major sites for the deposition of excess lipid and farmers often complain about the loss of weight this causes and the resultant economic loss.

The proximate composition of the fillets is clearly affected by the dietary oil level. The fish fed the high oil diets show decreased percentage ash and protein, although the absolute differences are only slight. However, the increases in percentage dry matter, lipid and energy are large. The change in dry matter and energy is mainly due to the increase in lipid in the fillets, which in fish weighing 340g rose from 10.3% in the fish fed the low oil diet, to 11.8% in the fish fed the high oil diet. The increase in fillet lipid would have been mainly at the expense of the level of moisture in the fillet, as the protein and ash levels were only slightly affected.

The variation in the proximate composition of the fillets is also greater in the fish fed the high oil diet. Thus, for example, fillet lipid ranges from 8.4% to 14.2% in the low oil diet fed fish and from 9.7% to 17.0% in the fish fed the high oil diet.

This experiment has shown that increasing the level of oil in the diets of rainbow trout results in faster growing fish. In three months of this trial the fish fed a 26% oil diet were on average 39g heavier than those fed a 16% oil diet. This amounts to a 16% increase in growth following a 10% increase in dietary oil.

However, the fish fed the higher oil diets show increased fillet lipid levels and increased losses at dress out. The increased loss at dress out is known to be economically undesirable to the farmer, but the economic effects of increasing the growth rate compared to the increased loss at dress out were not calculated in this trial. It could be that the reduced time taken to grow the fish to market size would compensate for the increased loss of weight at dress out.

The effect of increasing the fillet lipid level may have important effects on eating quality. Manipulating the fillet lipid level by starving the fish has been shown to effect the odour (Johansson and Kiessling, 1991) and the texture and flavour of rainbow trout (Johansson and Kiessling, 1991; Wiseman, 1993). Kestin *et al.* (1995b) showed some effects of fillet lipid levels on the eating quality of cooked rainbow trout. In a large population of rainbow trout, increases in the fillet lipid level resulted in changes in texture and flavour and an increase in overall preference over the levels of lipid investigated.

The next chapter of this thesis will describe experiments investigating the effects of fillet lipid levels on the texture and flavour of both smoked and cooked salmon. This will allow the direct effects on the consumer of increasing dietary oil levels in fish to be determined.

Chapter 3

The Effect of Muscle Lipid Content on the Eating Quality of Smoked and Fresh Atlantic Salmon

3.1 Introduction

The eating quality of a piece of flesh is affected by its composition. Kestin *et al.* (1995b) showed that the eating quality of rainbow trout was significantly affected by the level of muscle lipid. Increasing the level of lipid in fresh cooked rainbow trout resulted in an increased overall liking for the product.

In the previous chapter, it was shown how the level of lipid in the fish can be altered. Raising the level of dietary oil results in a significant increase in muscle lipid between groups of fish. However, within each dietary group, a range of lipid contents was observed.

Both salmon and trout farming are relatively new industries compared with land-based agriculture. There has been little genetic selection for traits and many farms still introduce wild stock into the gene pool. This results in a great deal of variation in some of the characteristics within a population of the fish. Consequently the degree of variation within any one population is wide enough to allow studies on certain characteristics of the fish to be carried out using only that population. This can reduce or eliminate the potential effects of extraneous factors on the experiment. For instance, differences in water temperature or flow rate between tanks or cages may have large effects on fish characteristics.

The work described in this chapter aims to use the degree of variation within one population of Atlantic salmon to generate a range of muscle lipid contents. The fish are then either cooked fresh or smoked and the eating quality of the flesh determined. This allows the effects of the level of fillet lipid on the eating quality to be assessed.

3.2 Method

3.2.1 Fish

A large population of Atlantic salmon, with an average weight of approximately 2.3kg, was raised on a standard commercial diet for six months on the Marine Harvest McConnell Ltd. Feed Trials Unit (FTU) in Loch Eil, Scotland. All fish were of the same age, having been transferred from freshwater one year after hatching and grown in the sea for 26 months prior to the experiment. The fish were kept in a commercial size cage (25m x 25m x 25m) during the growth phase. From this population one hundred and twenty fish were randomly selected and placed in a trials cage (5m x 5m x 5m) on the FTU. During stocking, the fish were individually weighed and the fork length determined while anaesthetised using benzocaine solution (E1501, Sigma, UK). During growth the fish were regularly inspected and any grilse were removed from the cage.

3.2.2 Diets

The fish were allowed to recover from handling for one week and were then fed a diet which was based on the formulation for a commercial diet (table 3.2.1). The diet was manufactured by BOCM Pauls, and the pellets were cut through a 10mm diameter die— the size recommended for salmon of this size (Appendix 2).

Table 3.2.1 Diet composition (analysis by P. Beardsworth, Hoffmann-La Roche, Heanor, Derbyshire, UK). Basic diet ingredients were LT fishmeal, fish oil, wheat, anti-oxidant (BHT), vitamin and mineral supplements and astaxanthin.

Dry Matter	94.5%
Protein	42.6%
Oil	28.9%
Fibre	0.90%
Ash	8.87%
Astaxanthin	76.3ppm

The feed was stored in a chilled room (at approximately 10°C) until required at the FTU. One month's supply was sent to the farm at a time, where it was stored in a dry feed shed. This helped to keep the feed fresh and minimised the loss of vitamins and oxidation of oils within the diet.

The feed was offered to the fish according to BOCM Pauls' feeding tables, which were based on the size of the fish and the water temperature (Appendix 2). The average rate of feeding was approximately 1.0% of the body weight per day. The amount delivered was adjusted weekly to allow for expected growth.

3.2.3 Husbandry

Every four weeks all the fish were individually weighed and measured as before. The fish were starved for one day before each weighing to reduce the level of excreted ammonia in the water during the stressful handling period. During the weighing procedure, any fish which had matured— *i.e.* all the grilse— were removed from the trials cage. These fish could be identified by a change in colouration from silver to brown and by a roughening of their skin to the touch.

The fish were routinely treated with Aquaguard (Ciba-Geigy Agrochemicals, Cambridge, UK) against sea lice (*Lepeophthirus salmonis* and *Caligus* sp.) six weeks and ten weeks after the start of the trial. After eleven weeks of the trial hydrogen peroxide was used instead, as it was found to be more effective against the infestation. During the trial twenty-one fish died from various causes, but the sea lice infestation, followed by secondary infections, was a major cause of mortality.

3.2.4 Slaughter and Processing

For seven days before the designated slaughter date the fish were deprived of food — a commercial procedure designed to clear the gut of food. The fish were then slaughtered on September 25th 1995. During the slaughter process the fish were crowded by lifting the bottom of the net cage up. The fish were then netted out by hand and stunned by a blow to the head with a priest — a 60cm long polypropylene club. The gills on one side of the fish were cut with a sharp knife and a tag identifying each fish attached to the gills on the other side of the head. The fish were then placed in a bin of ice slurry and allowed to bleed out. All the fish were placed in the same bin, which was occasionally stirred to ensure an even temperature throughout.

After slaughter, the bin was taken to the Marine Harvest McConnell processing unit at Blar Mhor, Fort William. The fish were individually weighed and measured, eviscerated and reweighed. They were then placed in polystyrene boxes (Lochaber Box Ltd., Fort William, Scotland) in batches of seven with ice packed around them and left until rigor had set in and resolved. During this period the fish were transported to Pinney's of Scotland smokehouse at Brydekirk, Scotland, where they were stored in the boxes in a room chilled to 4°C.

About 48 hours after slaughter, the fish were removed from the ice and filleted. Each fillet was tagged so as to be identifiable with the original fish. A 60g sample of flesh was removed from the right fillet of each fish (figure 3.2.1) and frozen. The right fillets were then individually vac-packed and frozen at -40°C. The left fillets were hand salted and left flat on racks for twenty hours to allow water to be drawn out of the flesh and the salt to penetrate. After this the fillets were rinsed in fresh water and placed flat on racks to drip dry before being moved into a kiln. They were cold smoked at 26°C for 14 hours using fumes from a burning mixture of 20% oak and 80% beech chippings. After smoking, the fillets were left for 4 hours and were then vac-packed and stored frozen at -40°C. All material was returned to Langford for storage and analysis.

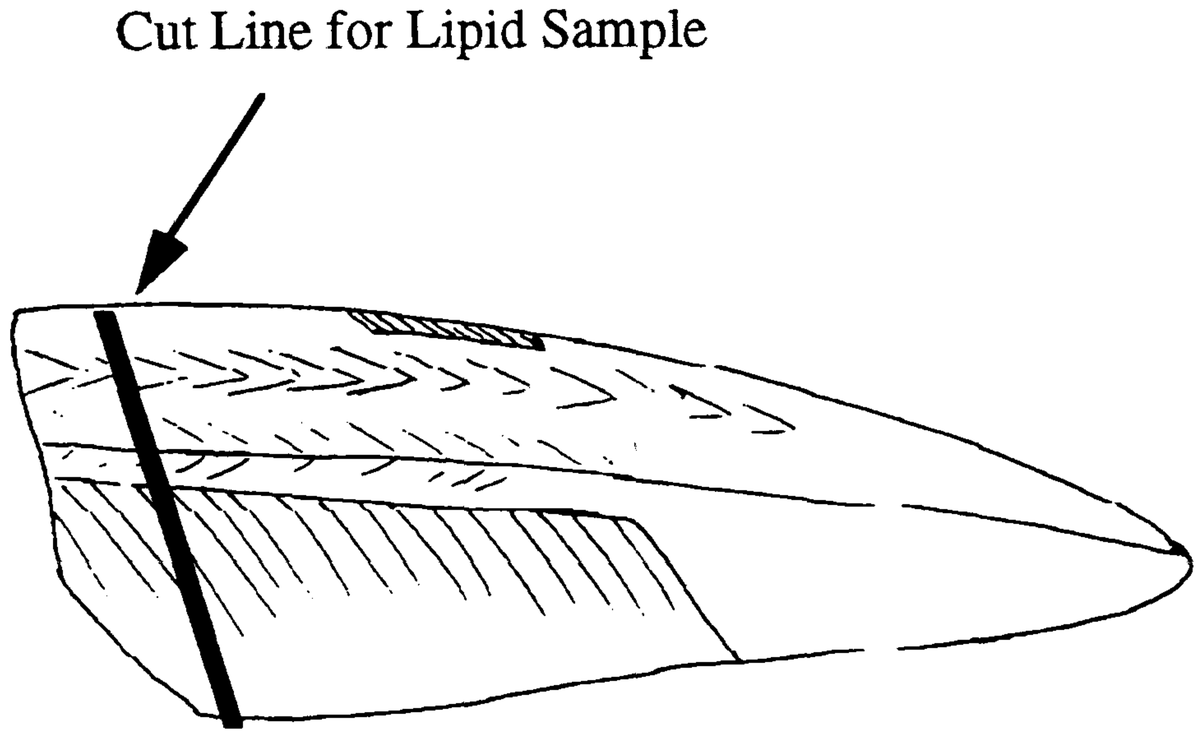

Figure 3.2.1: Position of lipid sample on the right fillet.

3.2.5 Lipid Analysis

The 60g samples of flesh were thawed and the skin and red muscle trimmed off, as were the lipid deposits at the dorsal section and the lower part of the belly flaps (figure 3.2.2). The remainder of each sample — approximately 40g — was then blended to a smooth paste using a Moulinette blender (Moulinex, UK). Two 5g portions of this paste were then analysed for total lipid content using a CEM AVC80

moisture and lipid analyser (CEM, UK). The machine was run on 95% power for three minutes to determine the moisture and on 100% power for two minutes to determine the lipid content after extraction with dichloromethane (Rathburns, Walkerburn, UK).

The results of the analysis were plotted on a graph to show the distribution of lipid content within the population. Four groups of lipid content were chosen, so that there were at least twenty fish in each group. The paired fillets — fresh and smoked — from these fish were to be used for taste panel analysis to determine the effect of lipid content on the eating quality of smoked fish (left fillet) and fresh fish (right fillet).

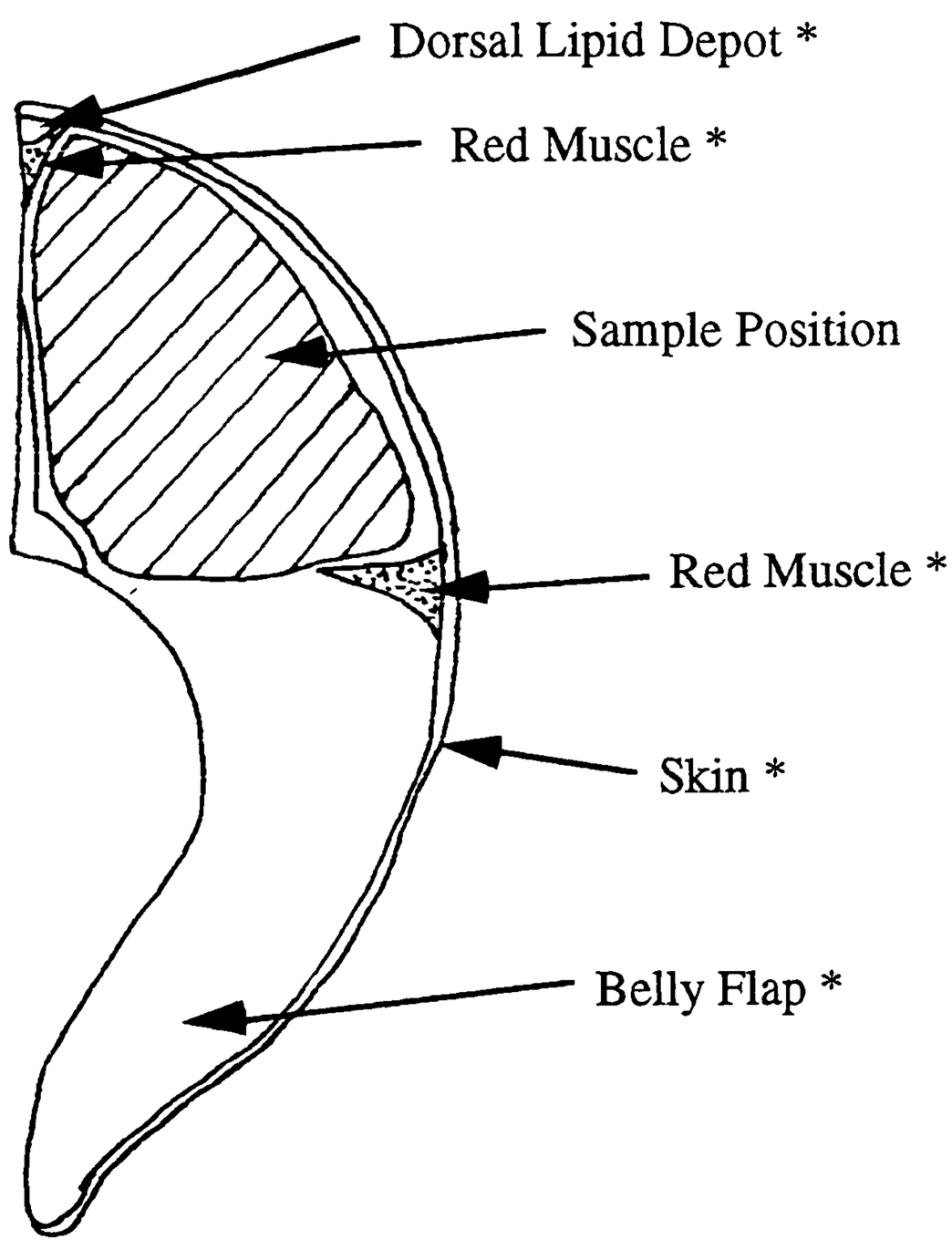

Figure 3.2.2: Position of lipid sample in the fillet taken from just behind the operculum. Tissues marked * were trimmed off the sample.

3.2.6 Taste Panelling

The taste panellists were a group of ten people selected for their ability to differentiate between grades of textures and flavours according to BS5929 part 4 (1986). Spare fillets, not allocated for panelling, were used for training the panellists. This enabled them to determine a list of attributes for which they could then assess the ratings for each sample.

The panels were set up in order of increasing lipid content, *i.e.* so that the first panel contained the fillets with the lowest lipid content from each group and the last panel had the highest. Each panel had a sample from one fillet from each group— *i.e.* there were twenty panels of four samples for the smoked and another twenty for the fresh fillets. Each fillet was prepared in the same way so that presentation would have no effect on the results.

The smoked fillets were trimmed to remove the outer smoked layer. The skin, red muscle and lipid depots were then cut off as for the lipid content samples, leaving just the white muscle. This was then cut into ten equal-sized samples along the length of the fish from the anterior end to beneath the posterior end of the dorsal fin (figure 3.2.3). The samples were kept in order according to position and each panellist always received a sample from the same position. This was to minimise any positional effects that may have occurred along the length of the fish. Each sample was placed in an individual clean glass petri dish and stored at room temperature until ready for panelling.

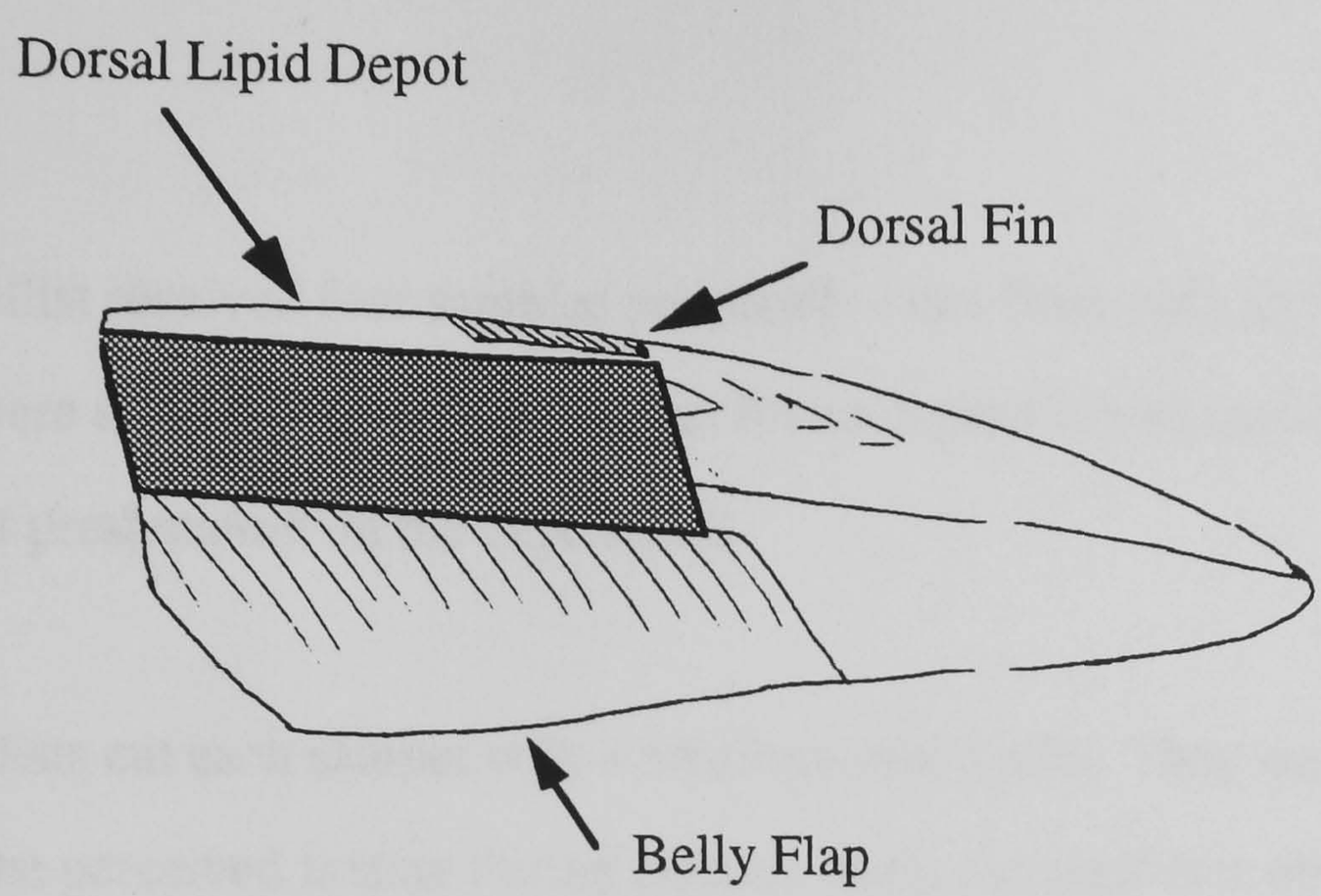

Figure 3.2.3: Region of flesh from the fillet used for taste panelling (shaded).

The fresh fillets were wrapped individually in aluminium foil and cooked in computer-controlled ovens at 180°C. The internal temperature of the fillets was determined using thermocouples attached to a digital temperature readout (Comark, UK). When the internal temperature had reached 65°C in the thickest part of the fillet, without exceeding 70°C in the rest of the fillet, the fillet was removed from the oven.

Once cooked the fillets were trimmed to remove the skin, red muscle and dorsal and belly lipid depots. The remaining white muscle was cut into ten samples as with the smoked fish. These were placed in individual clean glass petri dishes and stored at 65°C until ready for panelling, when they were presented to the panellists on a hot plate at 65°C.

The taste panel room was an isolated room with no windows. Extractor fans kept the air fresh so that no odours collected in the room which might have confused the results. The room was lit with red light so that the appearance of the fish did not affect the results. During panelling the panellists had access to plain bread rolls and

filtered water, so that they could clear the taste of previous samples from their mouths.

Each panellist received four samples per panel— one from each lipid group. The samples were arranged in different orders for each panellist so that there was no effect of order of presentation on the experiment.

The panellists cut each sample with a stainless steel knife. They were asked to describe the perceived texture during cutting, using the attributes obtained from the training sessions. The panellists then placed part of the sample in their mouths and described the texture of the first bite. The sample was chewed and again the texture was described. The panellists then took a second piece of the sample and described the flavours perceived on chewing, again using the attributes agreed on during the training sessions. If it was felt that a new texture or flavour descriptor was required to aptly describe a sample then the panellists were able to record that as well.

Rating of the attributes was carried out using line scales. Using a computer, panellists had to move a cursor along a line to a point which they felt best described the sample, where one end of the line was a rating of 0 (none) and the other 100 (maximum) for each descriptor. The information was then stored for later analysis.

3.2.4 Data Analysis

The results from the taste panellists were analysed using the statistics programme Genstat 5 release 3.1. The means for each descriptor for each sample were found and then the lipid groups compared using a one factor ANOVA for each descriptor. This allowed the effect of each lipid group on eating quality to be determined.

The individual mean ratings for each fillet were then plotted against the lipid content of the fillet and a regression line fitted— the line giving the best correlation coefficient to the data was plotted and this was generally found to be have a quadratic equation. The equation of the line and correlation coefficient were determined and the degree of significance calculated. This allowed the exact effect of lipid content on eating quality to be determined for both fresh and smoked fish.

3.3 Results

3.3.1 Fish and Environment

At stocking out the mean weight of the fish was 2.38 ± 0.043kg. During the ten weeks of feeding the fish grew well for the first six weeks, reaching a maximum average weight of 2.88 ± 0.048kg, before becoming infested with sea lice. The parasites, and the necessary treatments combined with the high water temperatures (figure 3.3.1) stressed the fish, resulting in reduced growth rates (figure 3.3.2) and some mortalities.

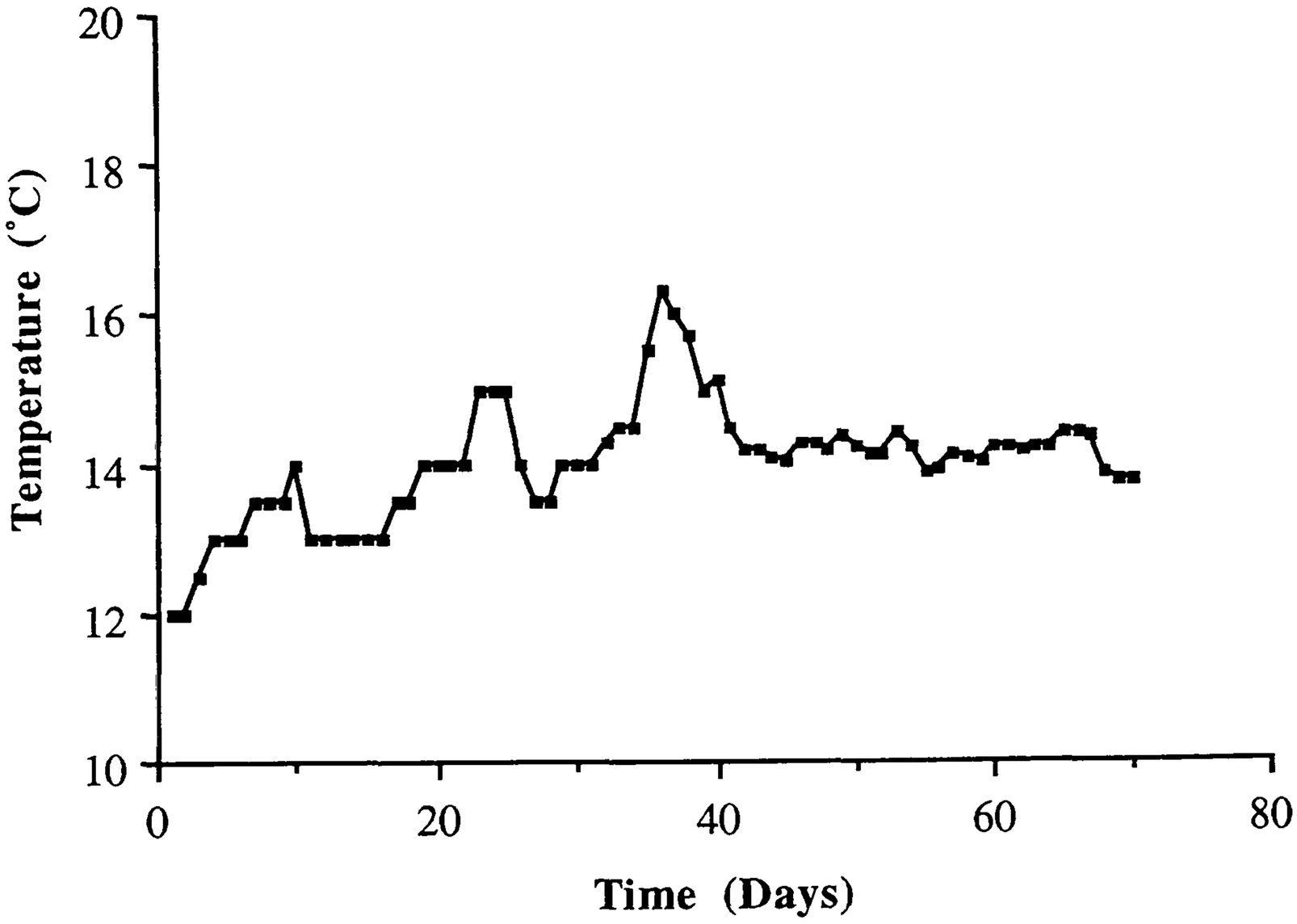

Figure 3.3.1: Average daily water temperature from the start of the trial to slaughter.

The water temperature increased during the trial (figure 3.1.1). Furthermore, the summer was very dry leading to high water salinity. This made the conditions ideal for the growth and breeding of the sea lice. It can be seen from the two graphs (figures 3.3.1 and 3.3.2) that the reduction in rate of growth occurred as the average

daily temperature reached its highest point, and that the mean weight decreased while the temperature remained high.

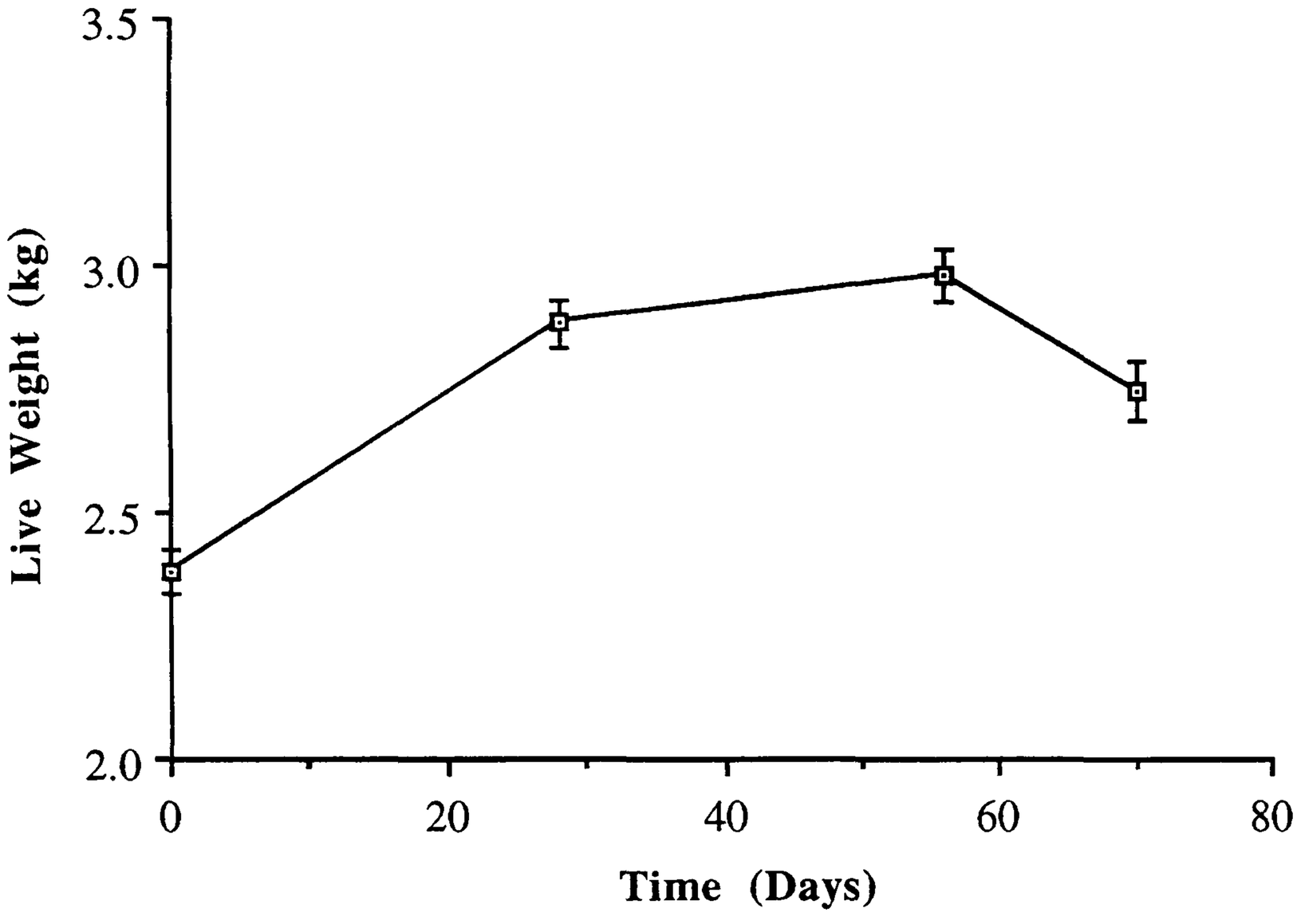

Figure 3.3.2: Growth of the fish during the trial — mean weight ± s.e.m.

At slaughter the mean live weight of the fish was 2.75 ± 0.061kg (range 1.24kg to 6.54kg), which was smaller than had been hoped for as market specifications for smoked salmon range between 3 and 5kg dressed weight. However, the trial was continued as the fish were not too small to smoke.

3.3.2 Lipid Analysis

The lipid content analysis showed a relationship between live weight and total lipid similar to that discussed in the previous chapter (figure 3.3.3). Total lipid content was worked out from the percentage lipid content of the sample and the live weight of each fish.

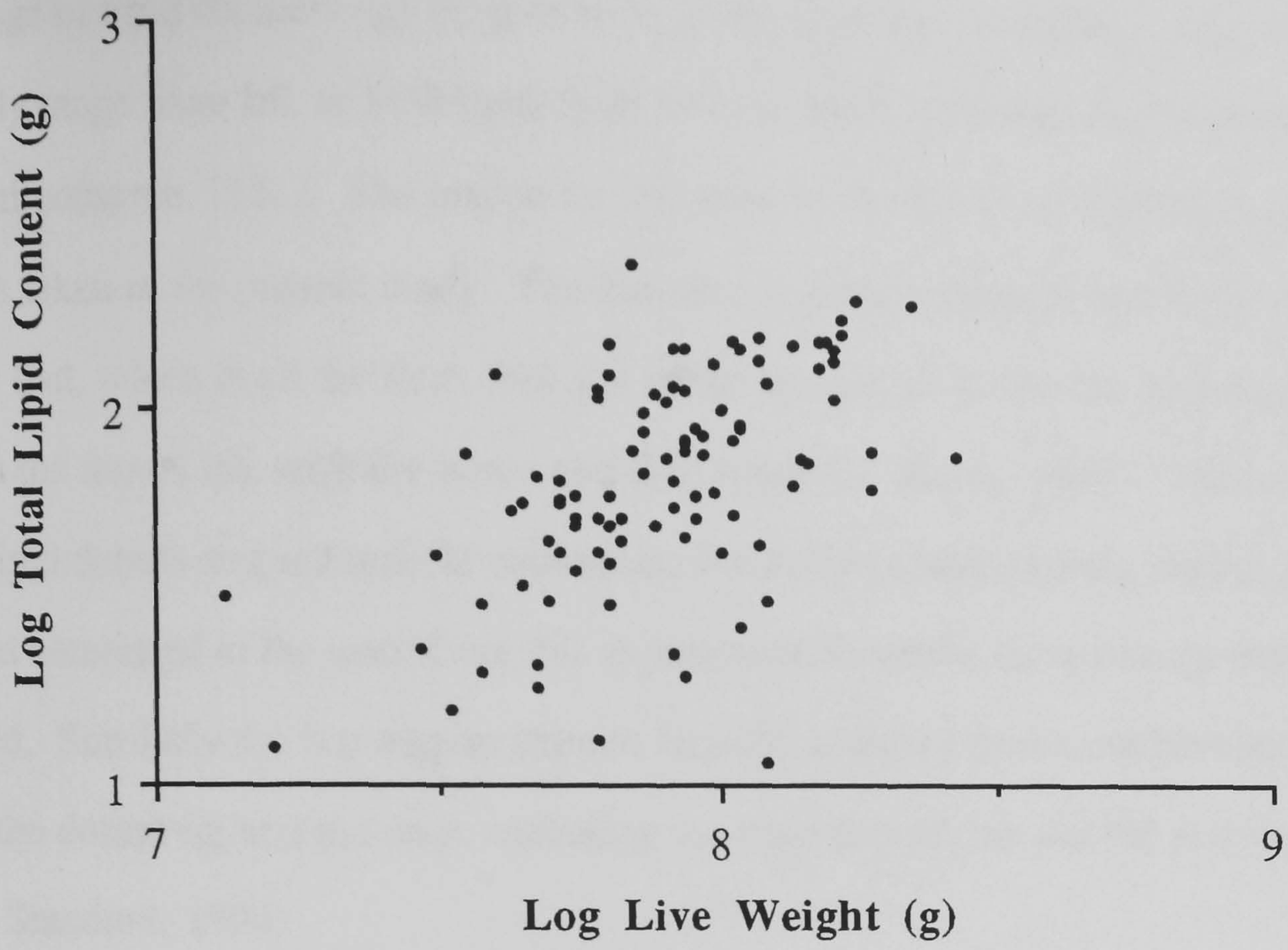

Figure 3.3.3: Log-log plot of total lipid content against live weight.

Using the per cent lipid content of the flesh samples, a plot of lipid content distribution within the population was obtained (figure 3.3.4). This showed a distribution of lipid content within the population, ranging from 2.9% to 10.7%.

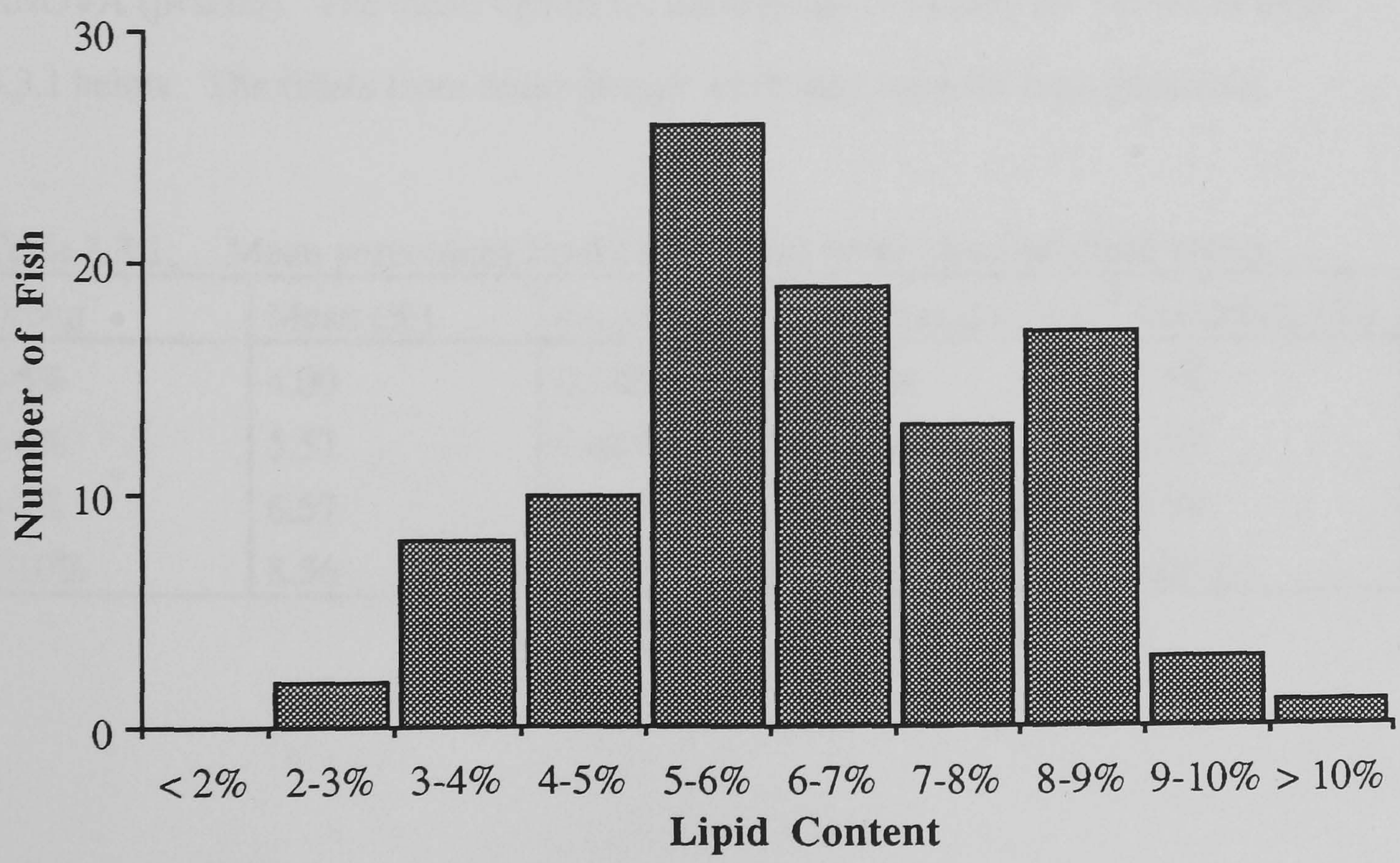

Figure 3.3.4: Lipid content distribution within the whole population.

The range of lipid contents can be seen to be lower than typical industry figures which currently range from 8% to 16% lipid (pers. comm. Mark Osborne, Acorn Smokeries Plc., Chilcompton, U.K.). The reason for this may be the anatomical position of the samples taken in the present study. The industry in Britain tends to use the Scottish Quality Cut, which is all the flesh (red and white muscle including the lipid depots) beneath the dorsal fin, with the bones and skin removed (Anon., 1995). The inclusion of the lipid depots and red muscle could raise the values obtained from the lipid analyses compared to the ones from this experiment, in which those tissues were removed. Similarly the Norwegian salmon farming industry uses a cut between the end of the dorsal fin and the anus, including the lipid depots, but not the skin or bones (Norsk Standard, 1994)

From the distribution of lipid content it was possible to split the population into four groups, each containing at least twenty fish. The groups were 2-5%, 5-6%, 6-7% and 7-10% lipid. Twenty fish were selected from these groups, so that the mean lipid contents of the groups were significantly different when tested with a one factor ANOVA ($p<0.05$). The mean values for each group of twenty are shown in table 3.3.1 below. The fillets from those groups were then used for taste panelling.

Table 3.3.1: Mean percentage lipid content and ranges for each lipid group.

Group	Mean (%)	s.e.m.	Minimum (%)	Maximum (%)
2-5%	4.00	0.143	2.89	4.98
5-6%	5.57	0.049	5.18	5.87
6-7%	6.57	0.063	6.08	6.99
7-10%	8.56	0.189	7.48	10.69

3.3.3 Smoked Fish

The preparation of the smoked fish for taste panelling was straightforward. The outer layer of smoked flesh, the skin and red muscle could be easily dissected from the white muscle, leaving a reproducible sample for taste panelling.

During the training sessions the taste panellists decided on the words that would be used for the texture and flavour attributes and their meanings are shown in table 3.3.2 and table 3.3.3 respectively. Each sample was rated for each attribute and at the end, each sample was rated for overall flavour and overall liking using a hedonic rating of 0 (minimum) to 100 (maximum).

Table 3.3.2: Texture attributes.

Texture On Cutting	*Firm*	The perceived force to cut the sample with a knife
	Clean-cut	Amount of sample not cut through cleanly (cut through middle of sample across line of fibres and assess number of fibres not cut)
Texture of Initial Bite	*Slimy*	Moist, slippery texture on the sample
	Oily	Feel of soft cooking fat
	Firm	The perceived force required to compress the sample using the molar teeth
Texture On Chewing	*Jellified*	Amount of gelatinous jelly
	Moist	The perceived degree of oil and/or water in the sample during chewing
	Firm	The perceived force required to compress the sample using the molar teeth.
	Dissolubility	Degree to which the sample melts in the mouth
	Cohesion	Degree to which the sample sticks together before swallowing
	Chewy	The total perceived effort required to prepare the sample to a state ready for swallowing

Table 3.3.3: Flavour attributes.

Flavour On Chewing	*Fishy*	Amount of fish flavour
	Salty	Taste on the tongue associated with salt
	Smoky	Taste on the tongue associated with smoke curing
	Sour	Acidic flavour
	Oily	Taste on the tongue associated with liquid fat
	Metallic	Taste of metal.

3.3.3.1 Texture of Smoked Salmon on Cutting

i) Firmness

The *firmness* of the sample under the knife was rated from 0 (very soft) to 100 (very hard). The mean rating for each fillet from the ten panellists was calculated. The four groups were then compared using a one factor ANOVA (table 3.3.4).

Table 3.3.4: Mean ratings for *firm* texture on cutting. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	44.8 ^{a}	40.2 ^{b}	37.8 ^{bc}	37.0 ^{c}	1.56	<0.001

There was a significant effect of the groups on the *firmness* ($p<0.001$) and as the lipid content of the group increased, the sample became less *firm* (table 3.3.4). However, the two highest groups had very similar means, implying that a minimum *firmness* level may have been reached or approached in the 6-7% lipid range.

The individual mean ratings were plotted against the individual mean lipid contents (figure 3.3.5). A regression line was plotted on the figure and the equation of the line and the correlation coefficient calculated.

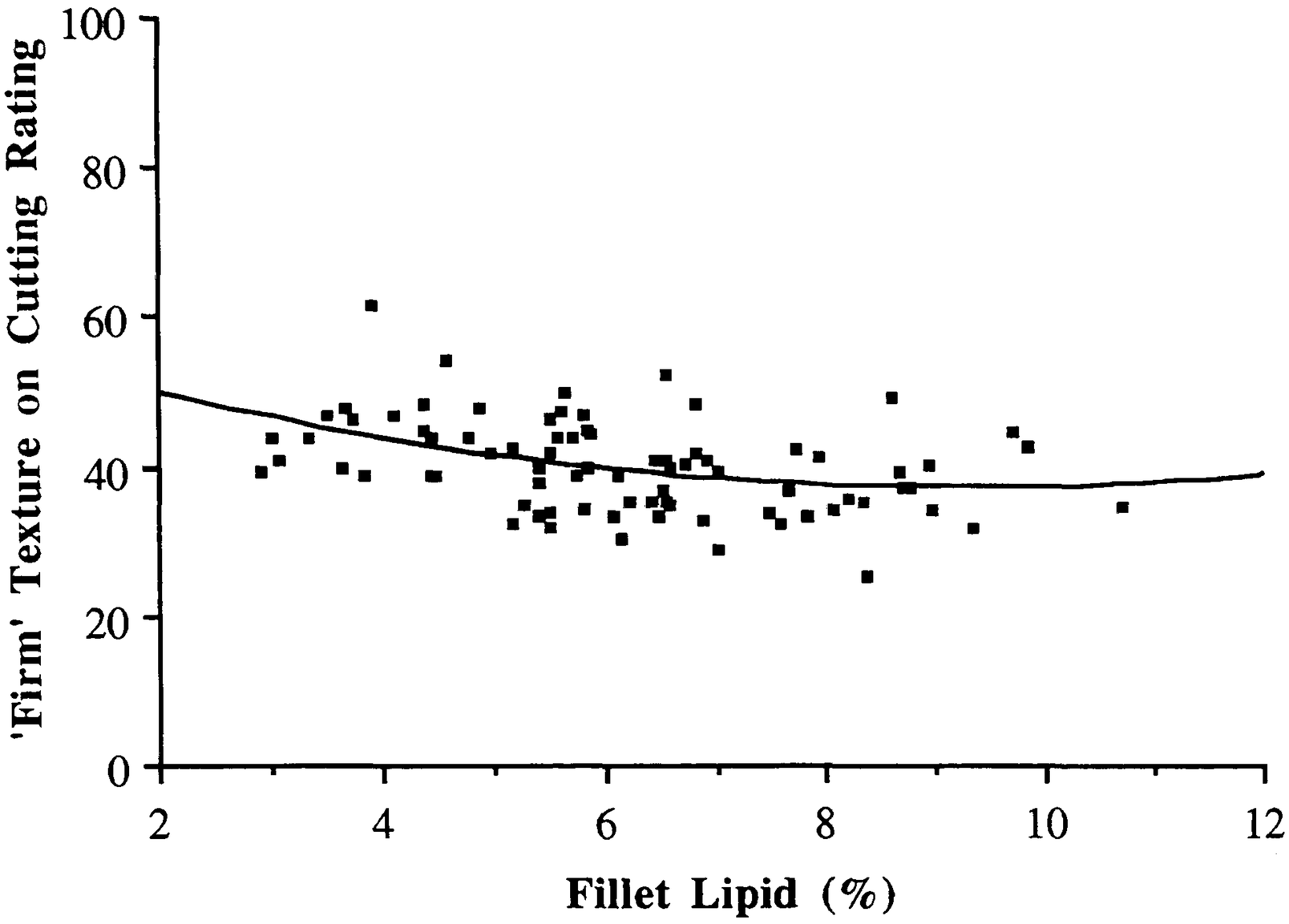

Figure 3.3.5: Relation between *firm* texture on cutting and lipid content.
$y = 0.25x^2 - 4.52x + 57.82$, $r^2 = 0.167$

From figure 3.3.5 it can be seen that there was a quadratic relationship between the lipid content of the fillet and the *firmness* of the flesh on cutting. With increasing lipid the *firm* texture decreased, but the decrease in *firmness* was less as the lipid increased further, indicating that a minimum *firmness* was approached. The relationship was significant ($r = 0.41$, $p<0.001$).

ii) Clean-Cut

The *clean-cut* texture was described as the number of fibres not cut through cleanly when the sample was cut in half along the line of the fibres. It was rated from 0 (all fibres cut through cleanly) to 100 (no fibres cut through).

The mean ratings for the fillets from each group were analysed using a one factor ANOVA. There was a significant effect of the lipid groups on the *clean-cut* ($p<0.01$), with fewer fibres being cut through cleanly with increasing lipid up to a maximum

point in the 5-6% group (table 3.3.5). Above this level there was no significant increase in the ratings.

Table 3.3.5: Mean ratings for *clean-cut* texture on cutting. Means with different superscript letters are significantly different ($p<0.05$). The probability in the final column is the probability that the lipid group did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	56.3 ^{a}	61.1 ^{b}	62.2 ^{b}	63.6 ^{b}	2.04	<0.001

The mean rating for each fillet was then plotted against its lipid content (figure 3.3.6). A regression slope was plotted and is shown in the figure with the equation and the r^2 value. A quadratic relationship was found between the lipid content and the rating for clean-cut (r=0.454) which showed that the correlation was significant ($p<0.001$). Thus the result confirmed that the rating for the *clean-cut* attribute increased with increasing lipid content up to about 5%. Above this point the rate of increase of the ratings slowed and started to drop above 9%.

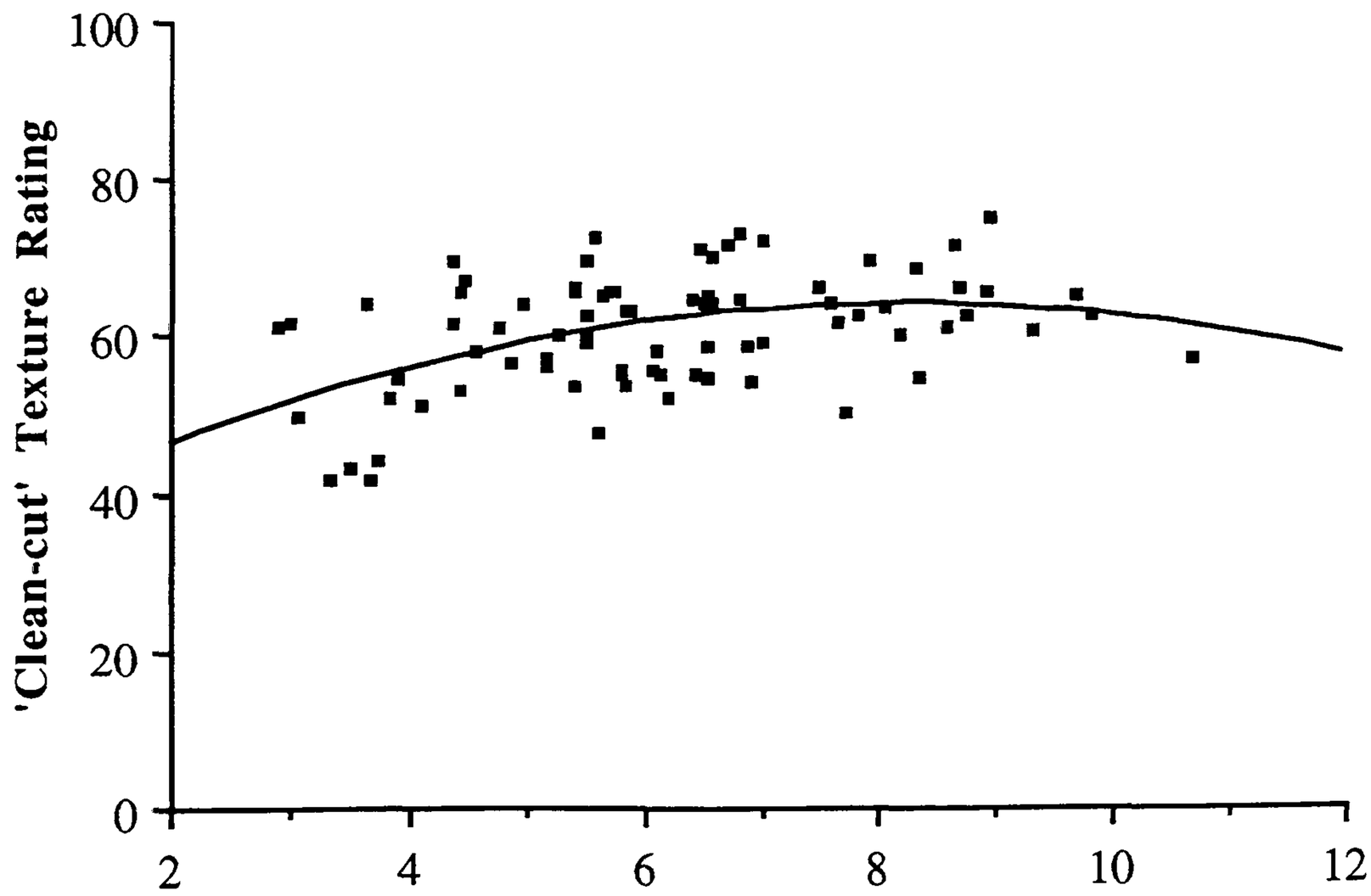

Figure 3.3.6: Relation between *clean-cut* texture on cutting and lipid content.
$y = -0.45x^2 + 7.38x + 33.35$, $r^2 = 0.206$

3.3.3.2 Texture of Smoked Salmon on First Bite

i) Slimy

The ratings of *slimy* texture were affected significantly by the lipid group (table 3.3.6). The ratings increased in the groups 2-5% and 5-6% and then were similar for the next two groups.

Table 3.3.6: Mean ratings for *slimy* texture on first bite. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	44.8 ^{a}	40.2 ^{b}	37.8 ^{bc}	37.0 ^{c}	1.56	<0.001

The mean fillet ratings for the attribute *slimy* were plotted against the fillet lipid content (figure 3.3.7). From this a regression line with a quadratic equation was plotted — the equation and r^2 value are shown on the figure. The r^2 value gave a significant ($p<0.01$) correlation coefficient of $r=0.370$.

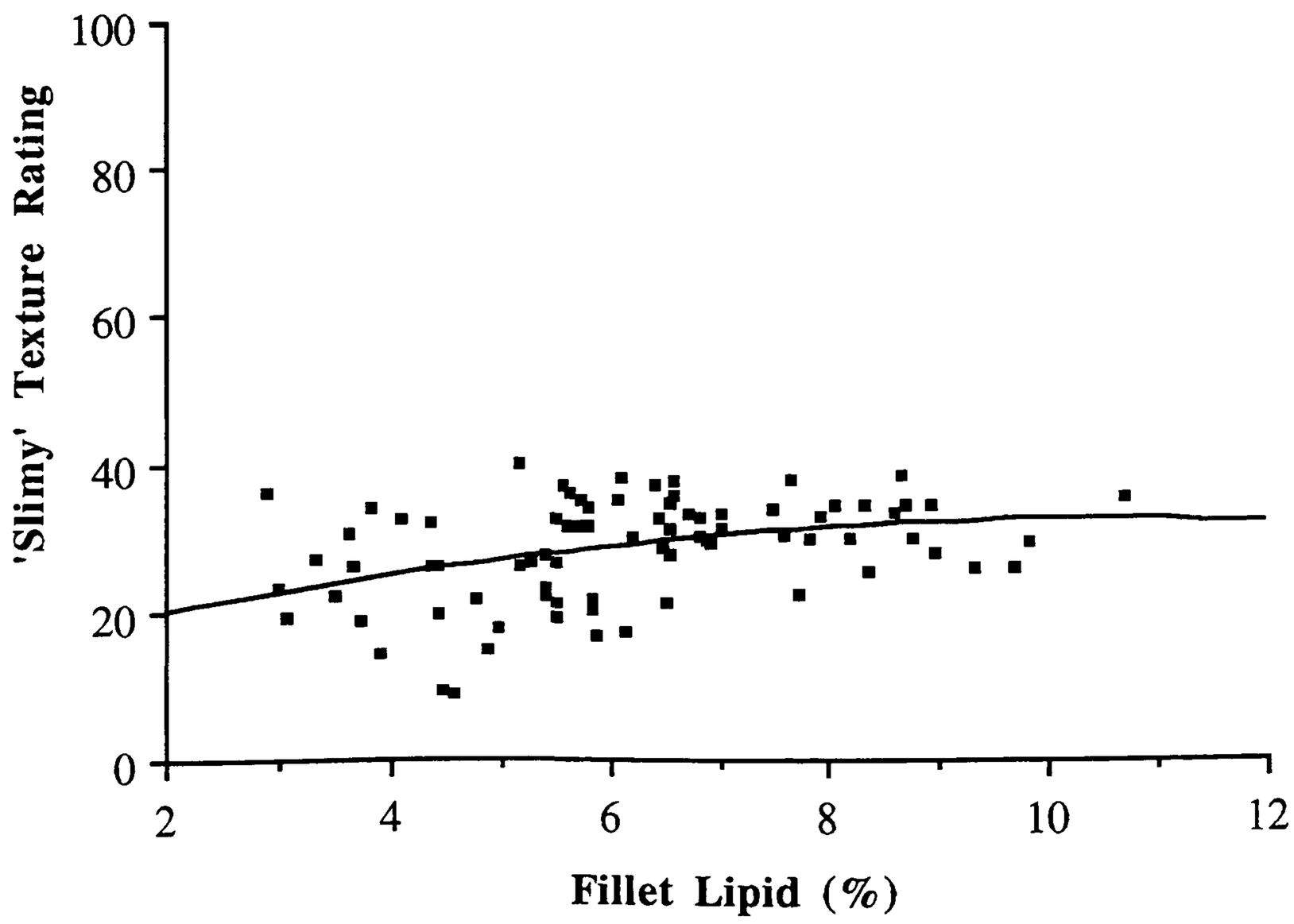

Figure 3.3.7: Relation between *slimy* texture on first bite and lipid content.
$y = -0.18x^2 + 3.68 + 13.14$, $r^2 = 0.140$

Figure 3.3.7 shows that at low lipid contents the *slimy* texture increased rapidly, but slowed as the lipid content approached 6-7%. A maximum rating may be reached by about 10%.

ii) Oily

The groups significantly ($p<0.001$) affected the *oily* sensation (table 3.3.7). Increasing lipid from 2-5% to 5-6% resulted in a significant difference, but there was no significant difference between the other groups and the mean ratings for the groups 6-7% and 7-10% were almost identical.

Table 3.3.7: Mean ratings for *oily* texture on first bite. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	34.2 ^{a}	37.8 ^{b}	39.8 ^{b}	39.1 ^{b}	1.42	<0.001

The mean ratings were plotted against the lipid content (figure 3.3.8). As before, a regression line with a quadratic equation gave the best fit to the data. The r^2 value for this gave a correlation coefficient of r=0.429, which shows a significant correlation ($p<0.001$). From figure 3.3.8 it can be seen that the *oily* texture ratings increased with increasing lipid to about 6%, when the rate of increase slowed, reaching a maximum at about 9%. This confirmed the results observed from the group ratings, which suggested that a maximum rating had been reached.

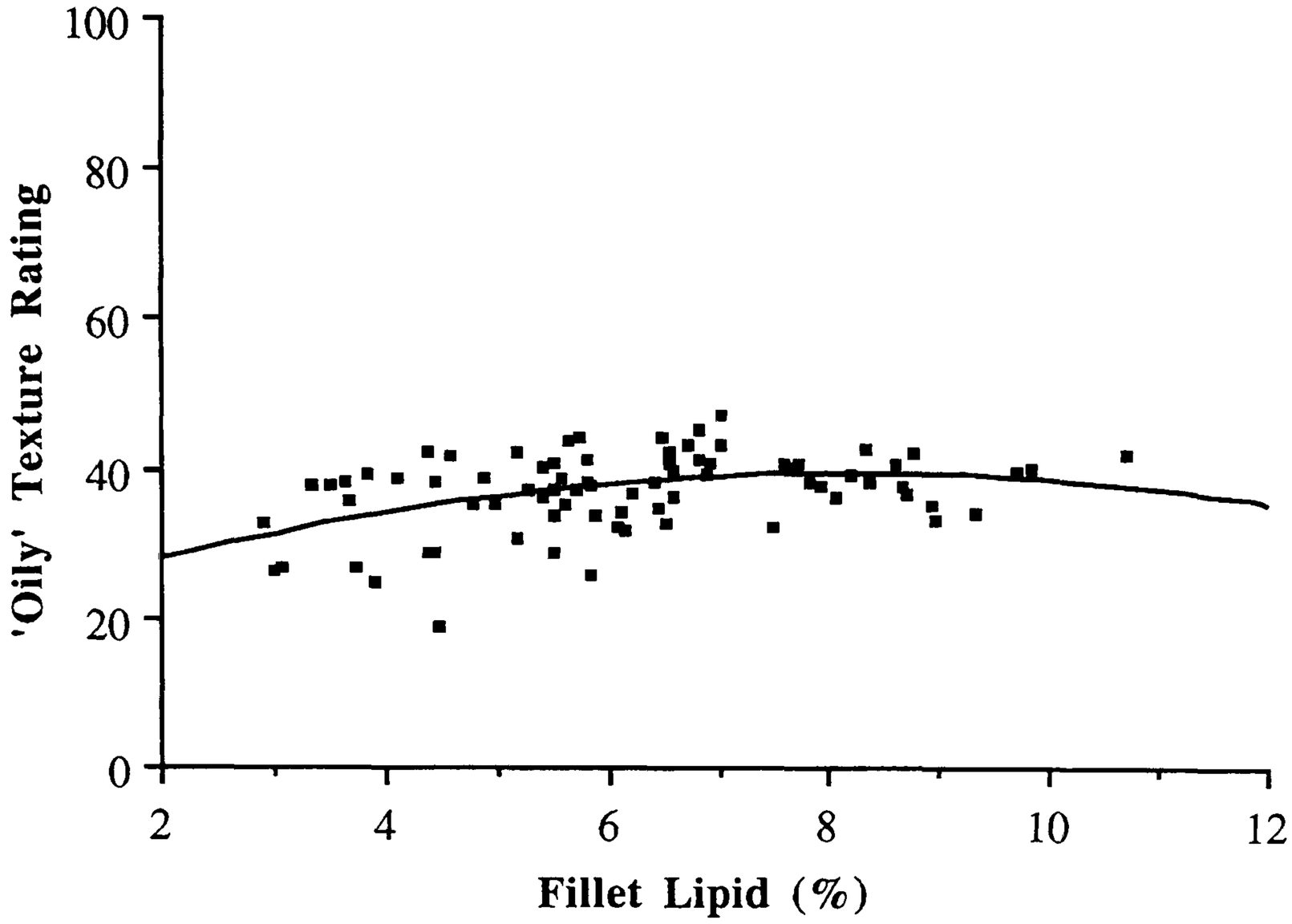

Figure 3.3.8: Relation between the *oily* texture on first bite and lipid content.
$y = -0.29x^2 + 4.83x + 19.24$, $r^2 = 0.184$

iii) Firmness

The mean ratings for each group show that the *firm* texture of the flesh was significantly ($p<0.001$) affected by the lipid group (table 3.3.8). *Firmness* decreased with the first two groups, but there was no difference between the groups 6-7% and 7-10% lipid.

Table 3.3.8: Mean ratings for *firm* texture on first bite. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	35.57 ^{a}	32.29 ^{b}	27.27 ^{c}	28.80 ^{c}	1.596	<0.001

A graph of the mean rating for each fillet was plotted against the fillet lipid content (figure 3.3.9). This confirmed that there was a relationship between *firmness* on the first bite and the lipid content of the fillet. The regression line, which had a quadratic

equation, had a correlation coefficient of r=0.445, which showed a significant relationship ($p<0.001$).

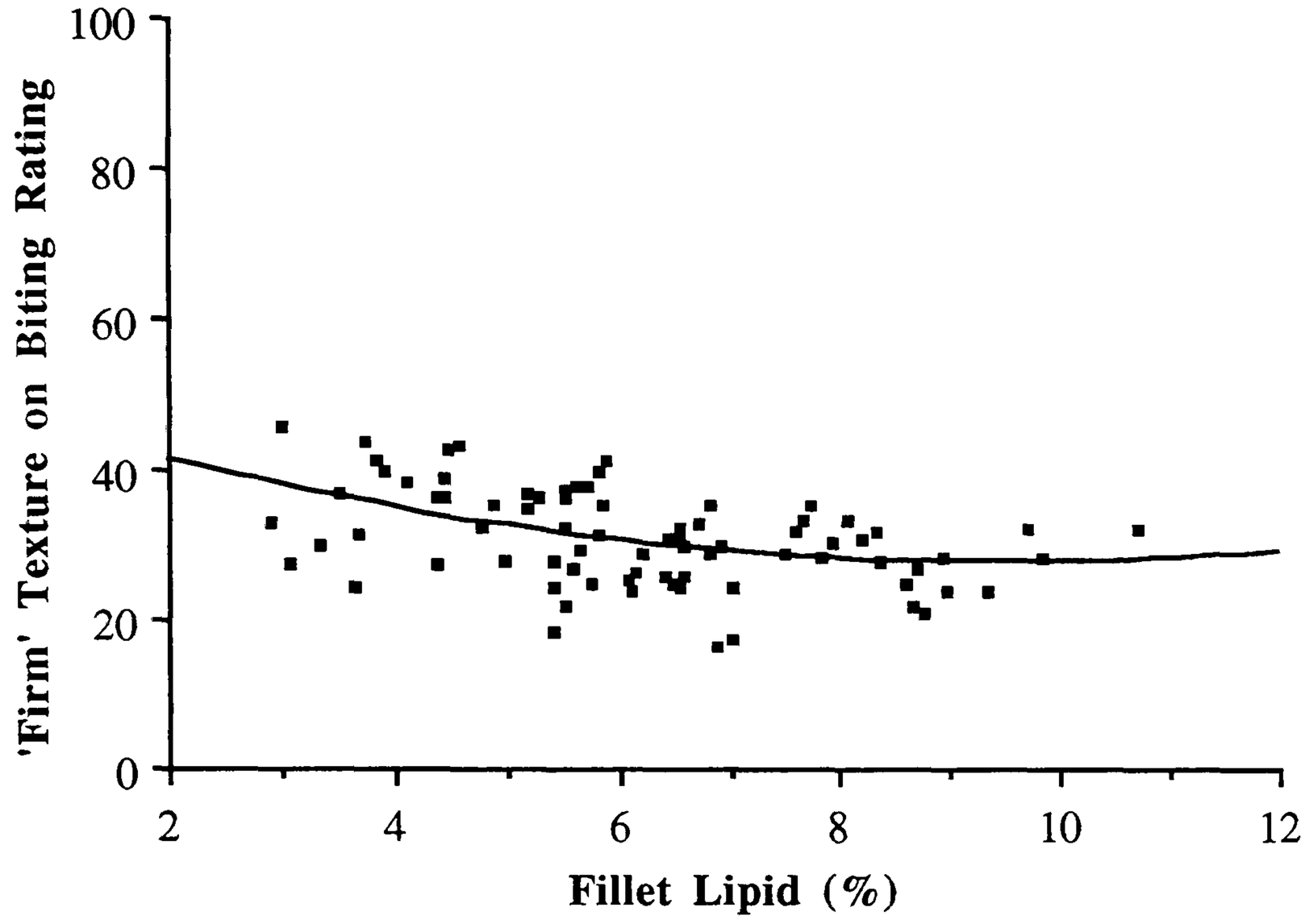

Figure 3.3.9: Relation between the *firm* texture on first bite and lipid content.
$y = 0.25x^2 - 4.71x + 49.75$, $r^2 = 0.198$

The ratings for *firmness* dropped with increasing lipid, reaching a minimum rating at about 8% (figure 3.3.9). This confirmed the results observed for the lipid groups, discussed above.

3.3.3.3 Texture of Smoked Salmon on Chewing

i) Jellified

Table 3.3.9 shows the mean ratings for each of the lipid groups for the texture attribute *jellified*. From this it can be seen that there was a significant effect of lipid group on the ratings for the *jellified* texture ($p<0.001$). The results show that there was no difference between the 2-5% and the 5-6% groups and the 6-7% and the 7-10% groups. However, the ratings for the first two groups were significantly lower than those for the second two.

Table 3.3.9: Mean ratings for *jellified* texture on chewing. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	38.0 ^{a}	38.4 ^{a}	44.1 ^{b}	43.3 ^{b}	1.63	<0.001

The mean rating for each fillet was plotted against the fillet lipid to observe the relation between the two (figure 3.3.10). A regression line with a quadratic curve was plotted on the graph and the correlation coefficient was calculated ($r=0.322$, $p<0.01$). The *jellified* rating increased throughout the lipid range investigated, although the rate of increase decreased with increasing lipid. This implied that a maximum *jellified* texture may be reached at a higher level of fillet lipid.

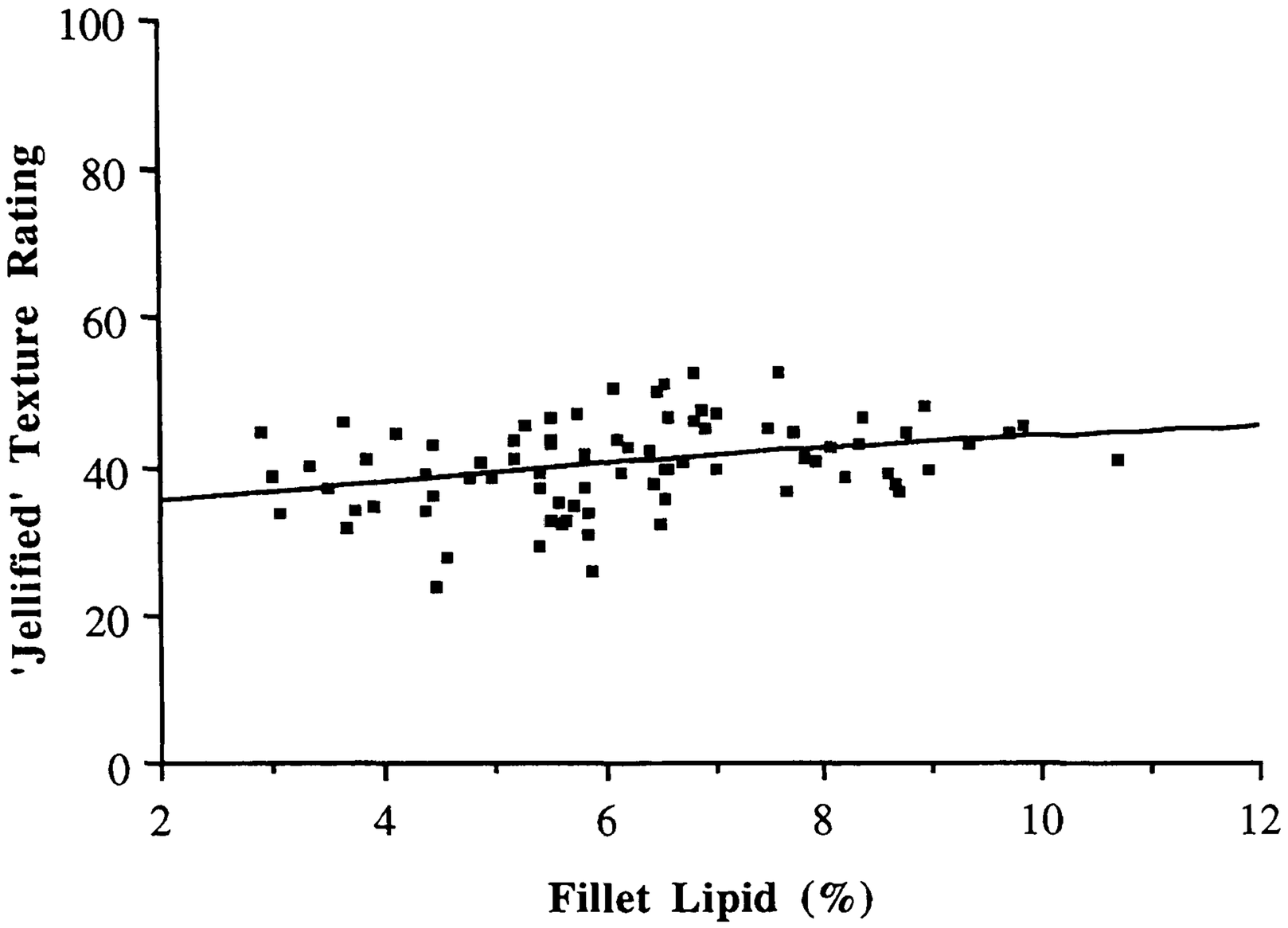

Figure 3.3.10: Relation between the *jellified* texture on first bite and lipid content.
$y = -0.04x^2 + 1.69x + 31.88$, $r^2 = 0.104$

ii) Moist

Table 3.3.10 shows the mean ratings of *moist* texture for each lipid group. From this it can be seen that there was a significant effect of the lipid groups on the *moist* texture ($p<0.001$) and that the *moist* sensation increased with increasing lipid for the first three groups, but there was no difference between the groups 6-7% and 7-10%.

Table 3.3.10: Mean ratings for *moist* texture on chewing. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	30.62 [a]	33.32 [b]	37.60 [c]	37.57 [c]	1.126	<0.001

Figure 3.3.11 shows the plot of lipid content against the mean fillet ratings for *moist* texture. A regression line with a quadratic equation was plotted on the graph and a correlation coefficient of $r=0.464$ was calculated ($p<0.001$).

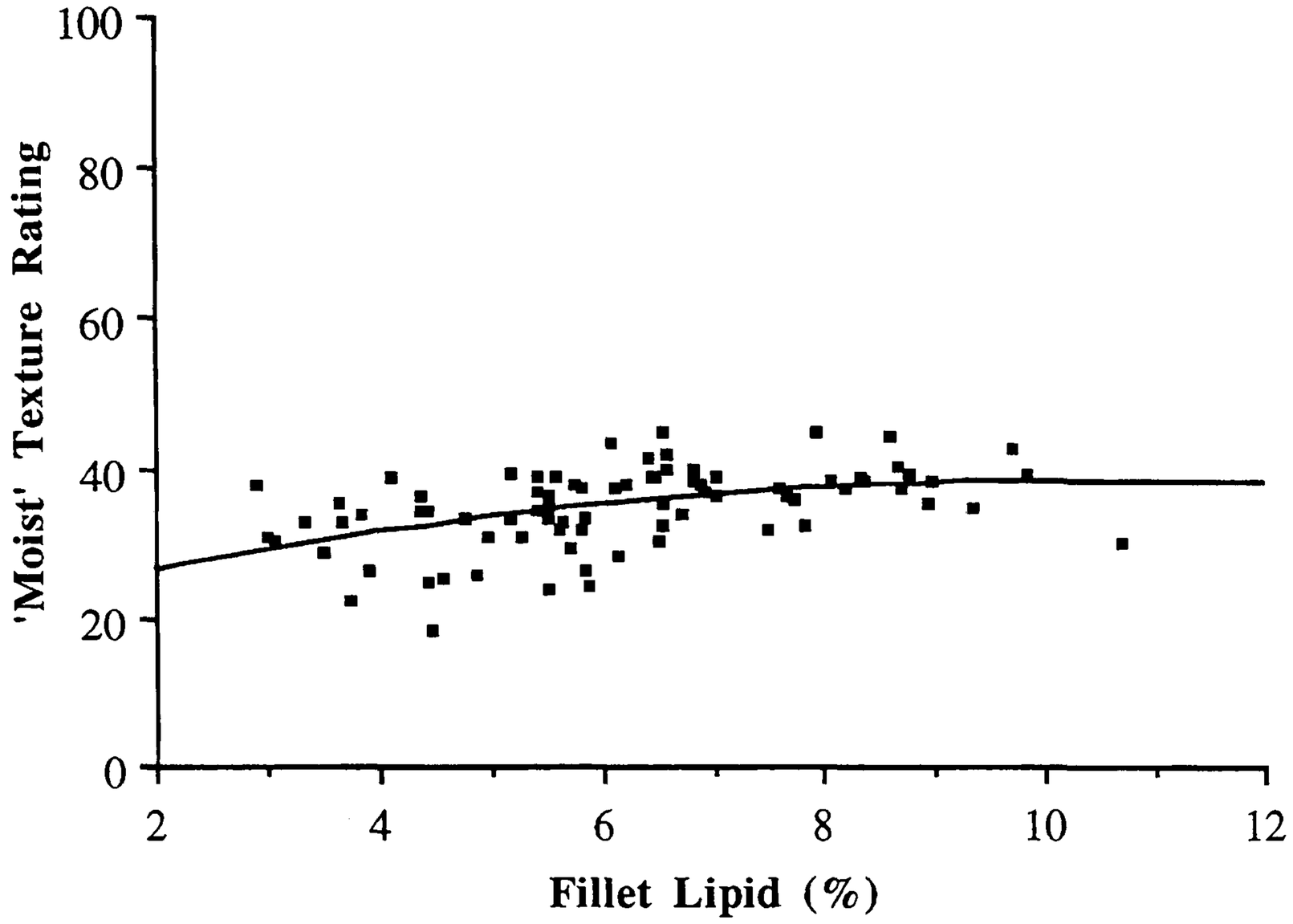

Figure 3.3.11 Relation between *moist* texture on first bite and lipid content.
$y = -0.16x^2 + 3.39x + 20.32$, $r^2 = 0.215$

The perceived *moistness* increased with increasing lipid up to 7-8%. A maximum rating was then reached at about 9%. This confirmed the results from the groups that there was little difference in the higher lipid levels.

iii) Firm

Firm texture was again used as an attribute for the sensation perceived on chewing. Table 3.3.11 shows the mean ratings for each lipid group. There was a significant effect of the groups on the ratings ($p<0.001$), with *firm* texture decreasing with increasing lipid as before. As had been observed for other attributes, there was no difference between the 2-5% and 5-6% groups and the 6-7% and 7-10% groups, but there was a significant difference between the first two groups and the last two.

Table 3.3.11: Mean ratings for *firm* texture on chewing. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	28.17 [a]	27.69 [a]	22.85 [b]	23.88 [b]	1.567	<0.001

The mean ratings were plotted against the fillet lipid content and a regression line fitted (figure 3.3.12). The equation of the regression line which best fitted the data was quadratic and the resulting r^2 value gave a significant correlation coefficient of $r=0.311$ ($p<0.01$).

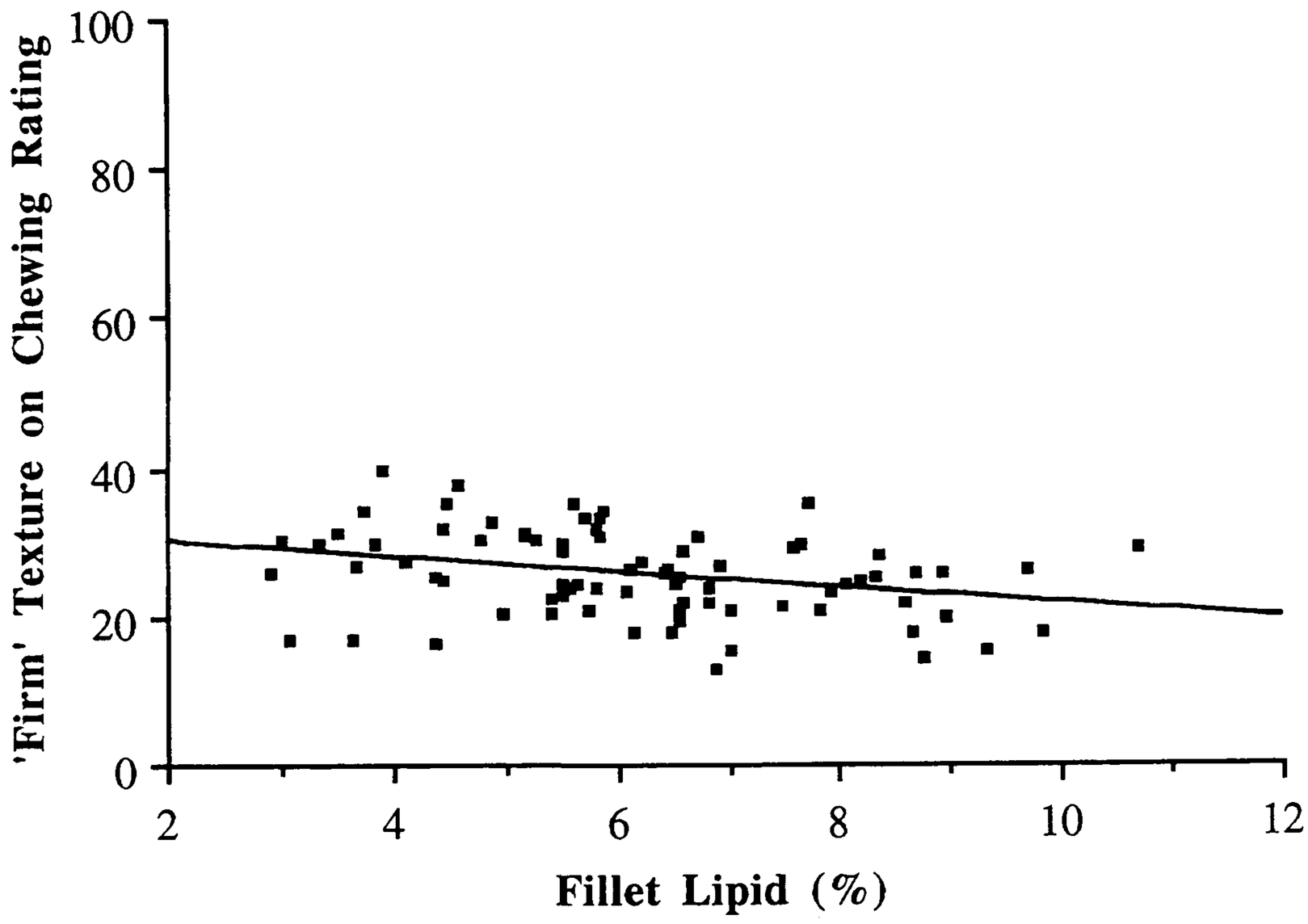

Figure 3.3.12: Relation between *firm* texture on chewing and lipid content.
$y = 0.006x^2 - 1.11x + 32.35$, $r^2 = 0.097$

Firm texture decreased with increasing lipid throughout the lipid range investigated (figure 3.3.12). As the equation for the regression line fitted was quadratic, a minimum *firmness* rating would eventually be reached, but this was not in the range investigated.

iv) Dissolubility

The ratings of *dissolubility* were significantly ($p<0.001$) affected by the lipid group (table 3.3.12). They increased between the groups 5-6% and 6-7% and 6-7% and 7-10%, although there was no difference between the 2-5% and 6-7% groups.

Table 3.3.12: Mean ratings for *dissolubility* on chewing. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	37.52 ^{ab}	36.54 ^{a}	40.89 ^{b}	43.29 ^{c}	1.748	<0.001

The plot of the mean ratings of each fillet for *dissolubility* were plotted against the lipid content (figure 3.3.13) and a regression line with a quadratic equation best fitted the data points. The correlation coefficient was calculated as $r=0.305$ ($p<0.01$). The perceived *dissolubility* of the samples increased with increasing lipid, although the rate of increase slowed over the range investigated.

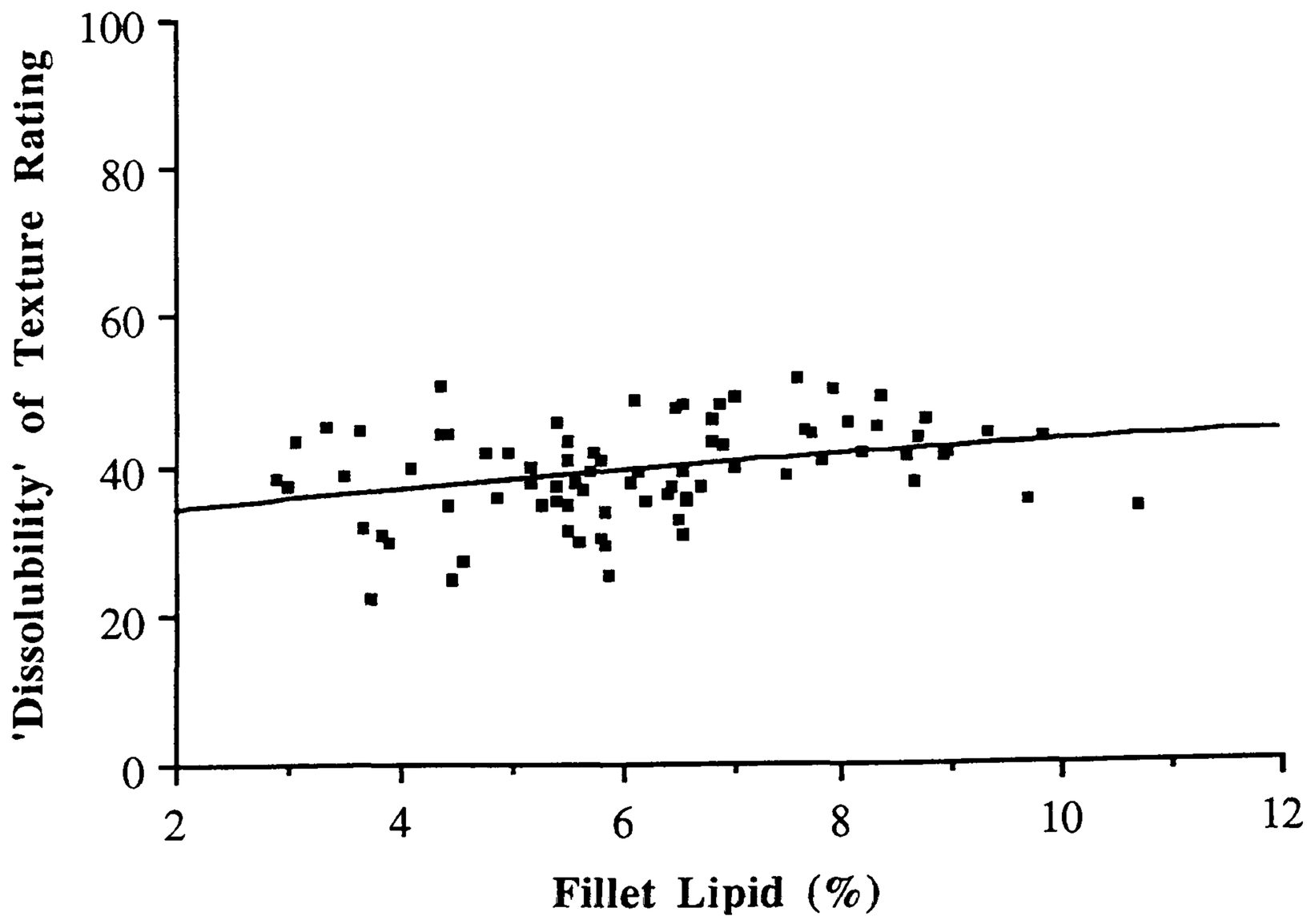

Figure 3.3.13: Relation between *dissolubility* on chewing and lipid content.
$y = -0.05x^2 + 1.71x + 30.74$, $r^2 = 0.093$

v) Cohesive

Table 3.3.13 shows the mean ratings for each lipid group. From this it can be seen that there was a non-significant trend towards the lipid group having an effect on the ratings (p=0.052).

Table 3.3.13: Mean ratings for *cohesive* texture on chewing. The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	31.94	32.67	30.08	28.36	1.703	0.052

Figure 3.3.14 shows the plot of the mean ratings for each fillet against the lipid content of the fillet. A regression line with a quadratic equation was fitted to the data, but the variation of the data was large, especially for the higher lipid levels. Thus the regression curve did not describe a significant relationship (p>0.05).

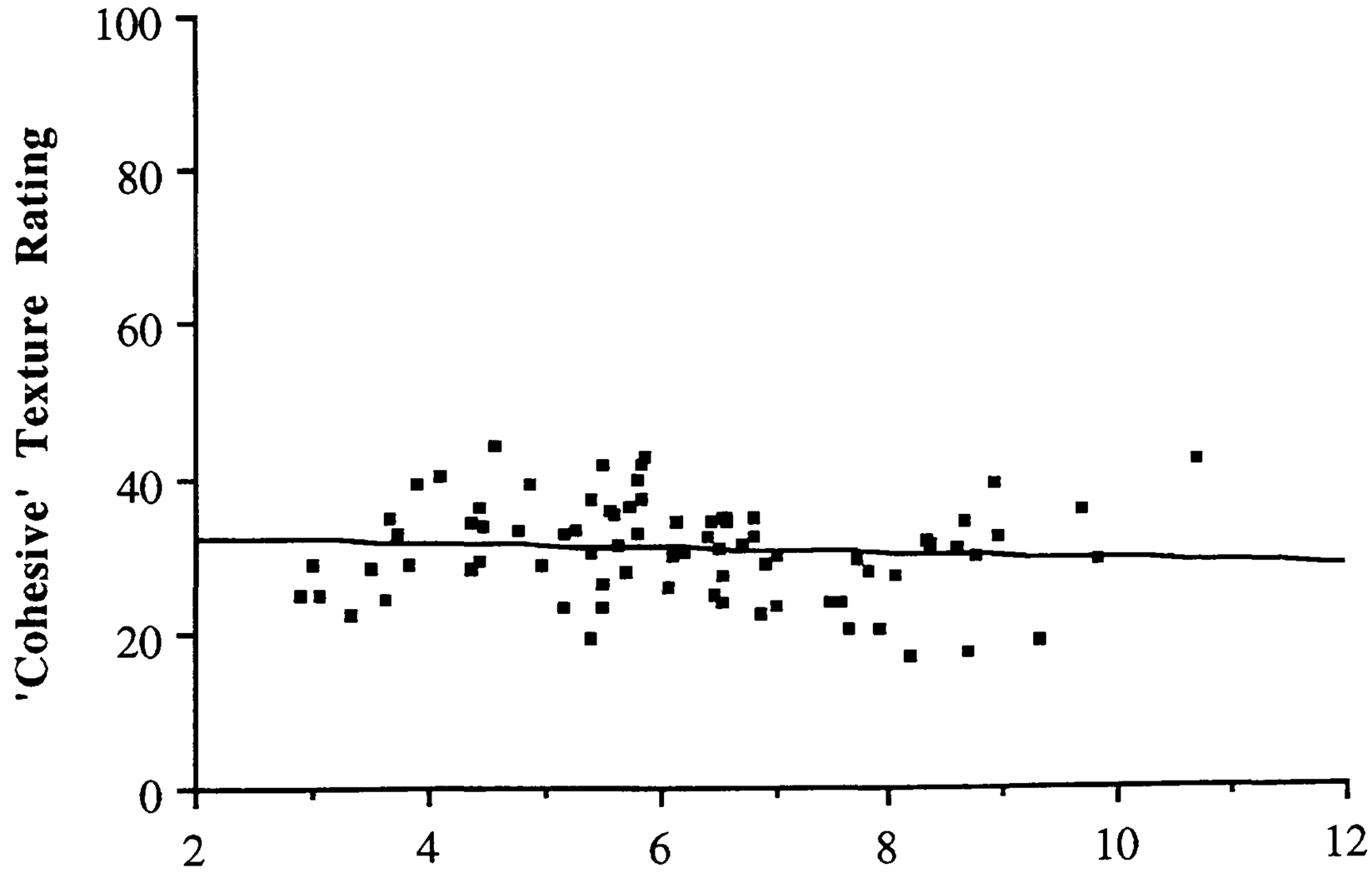

Figure 3.3.14: Relation between *cohesive* texture on chewing and lipid content.

$y = -0.004x^2 - 0.30x + 32.70$, $r^2 = 0.010$

vi) Chewy

The mean ratings of *chewy* texture for each lipid group are shown in table 3.3.14. From this it can be seen that there was a significant effect of the groups on the perceived *chewy* texture of the samples ($p<0.001$).

Table 3.3.14: Mean ratings for *chewy* texture on chewing. Means with different superscript letters are significantly different ($p<0.05$). The probability quoted in the last column is that of the lipid group not having an effect on the ratings.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	27.52 [a]	26.80 [a]	22.55 [b]	23.19 [b]	1.521	<0.001

The mean ratings for each fillet were plotted against the fillet lipid content (figure 3.3.15) and a regression line with a quadratic equation was fitted to the data. The correlation coefficient of $r=0.230$ was significant ($p<0.01$). The rating for *chewy* texture tended to decrease with increasing lipid until a lipid content of about 8% was reached, when the minimum *chewiness* was obtained.

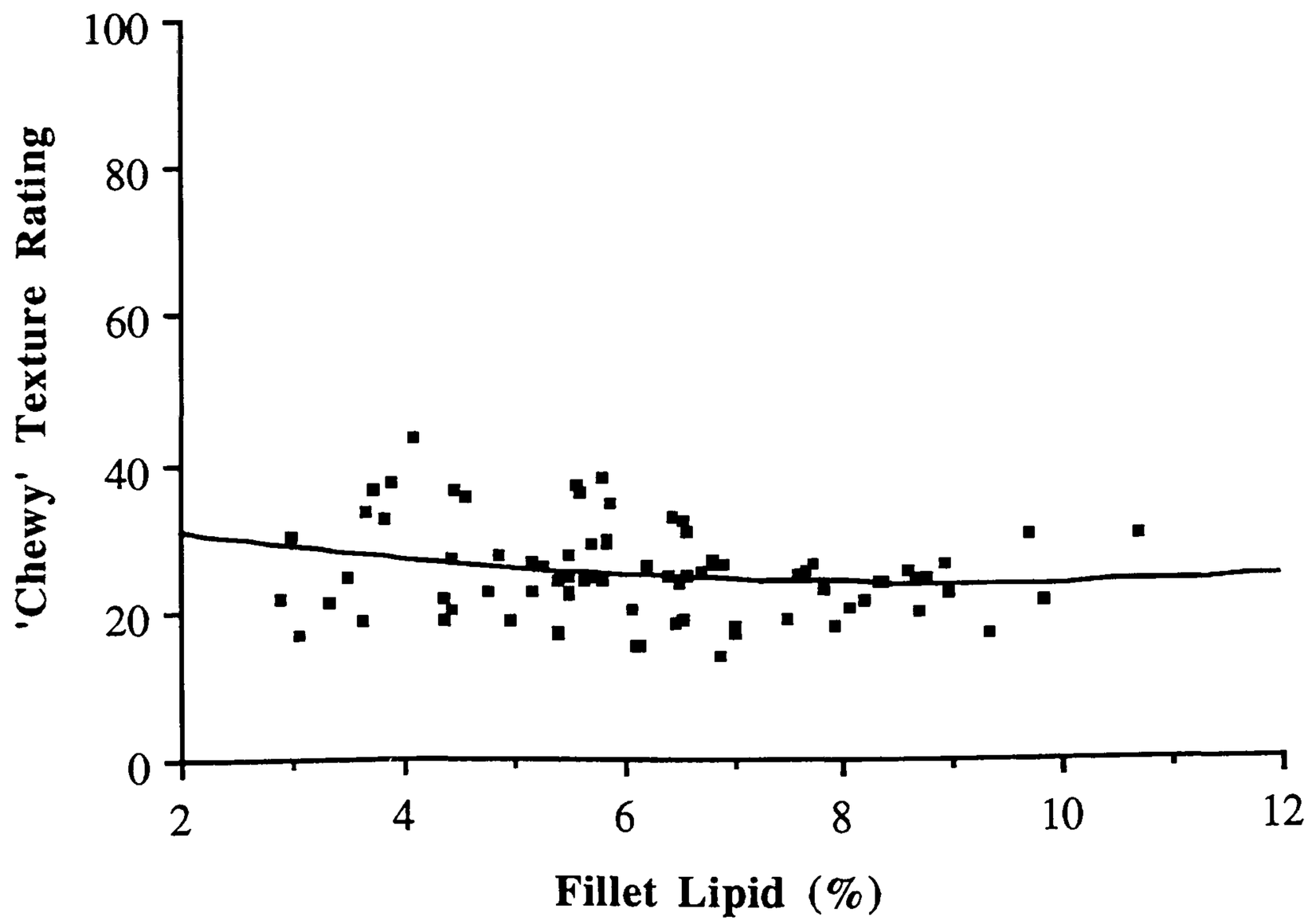

Figure 3.3.15: Relation between *chewy* texture on chewing and lipid content.
$y = 0.14x^2 - 2.51x + 35.16$, $r^2 = 0.053$

3.3.3.4 Flavour of Smoked Salmon on Chewing

i) Fishy

Table 3.3.15 shows the mean ratings for each lipid group. There was a significant effect of the groups on the degree of perceived *fishy* flavour ($p<0.001$). The 2-5% lipid group had significantly lower *fishy* flavour than the other groups, between which there were no differences.

Table 3.3.15: Mean ratings for the flavour attribute *fishy*. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	35.65 [a]	40.76 [b]	39.08 [b]	40.61 [b]	1.266	<0.001

The mean ratings for each fillet were plotted against the fillet lipid and a regression line fitted to the data points (figure 3.3.16). The correlation coefficient of this line was calculated as $r=0.324$, which indicated a significant effect of lipid content on the *fishy* flavour as perceived by the taste panellists ($p<0.01$). The *fishy* flavour rating increased with increasing lipid until approximately 6% lipid. Above 10% lipid the *fishy* flavour started to decrease again. This indicated that the maximum *fishy* flavour for the smoked fish was in the range 6-10%.

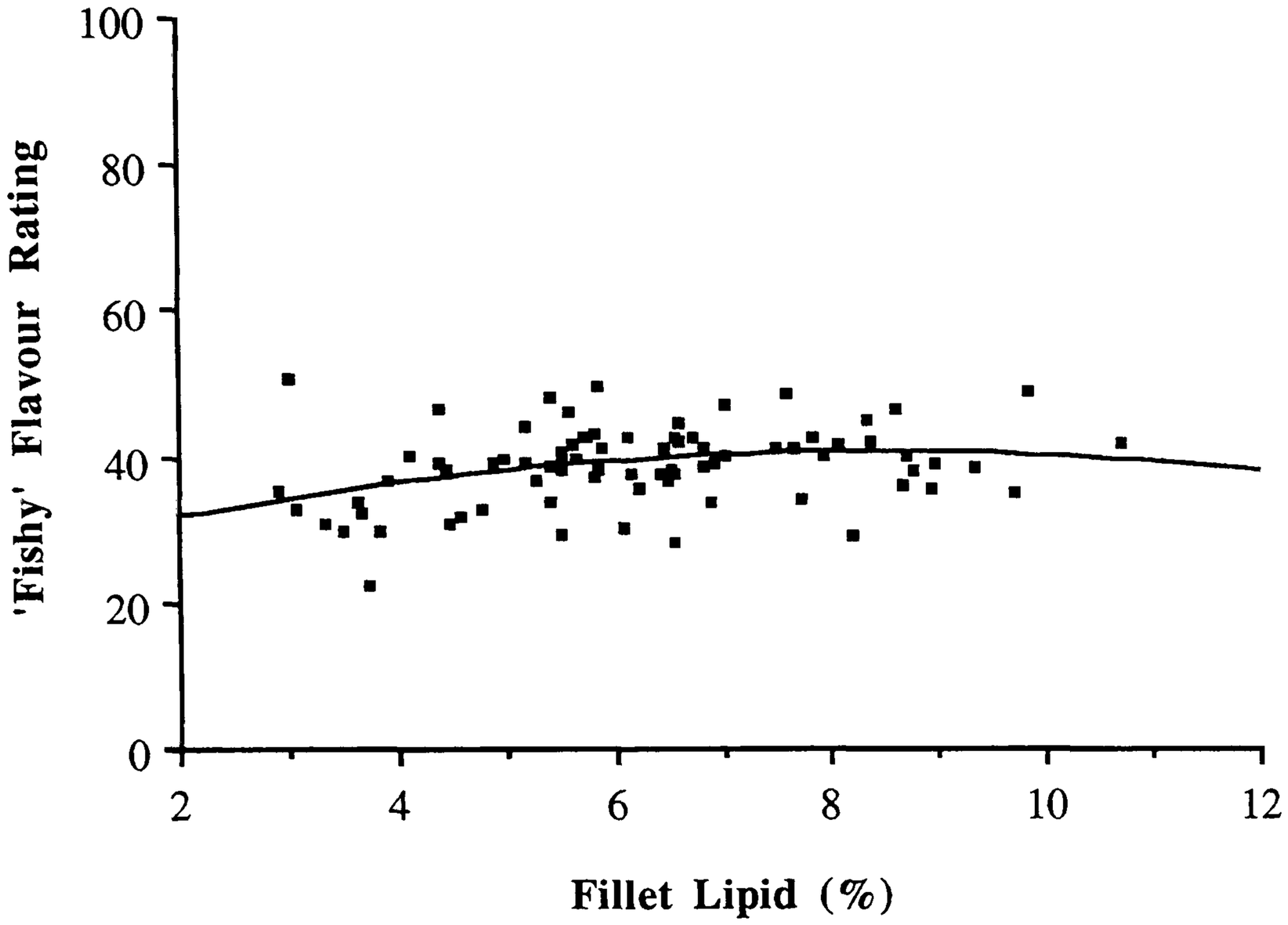

Figure 3.3.16: Relation between the *fishy* flavour and lipid content.
$y = -0.21x^2 + 3.63x + 25.24$, $r^2 = 0.105$

ii) Salty

Salty flavour was an important attribute as high ratings for *salt* may be aversive to the consumer. The mean ratings for each lipid group were calculated and compared (table 3.3.16). There was a significant effect of lipid content on the *salty* flavour ($p<0.001$). Each group was significantly different, with *salty* ratings decreasing as the lipid content increased.

Table 3.3.16: Mean ratings for the flavour attribute *salty*. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	61.45 ^{a}	54.68 ^{b}	49.87 ^{c}	45.42 ^{d}	1.788	<0.001

The mean ratings for *salty* flavour were plotted against the fillet lipid content and a regression line fitted (figure 3.3.17). The correlation coefficient of the line was $r=0.530$ ($p<0.001$).

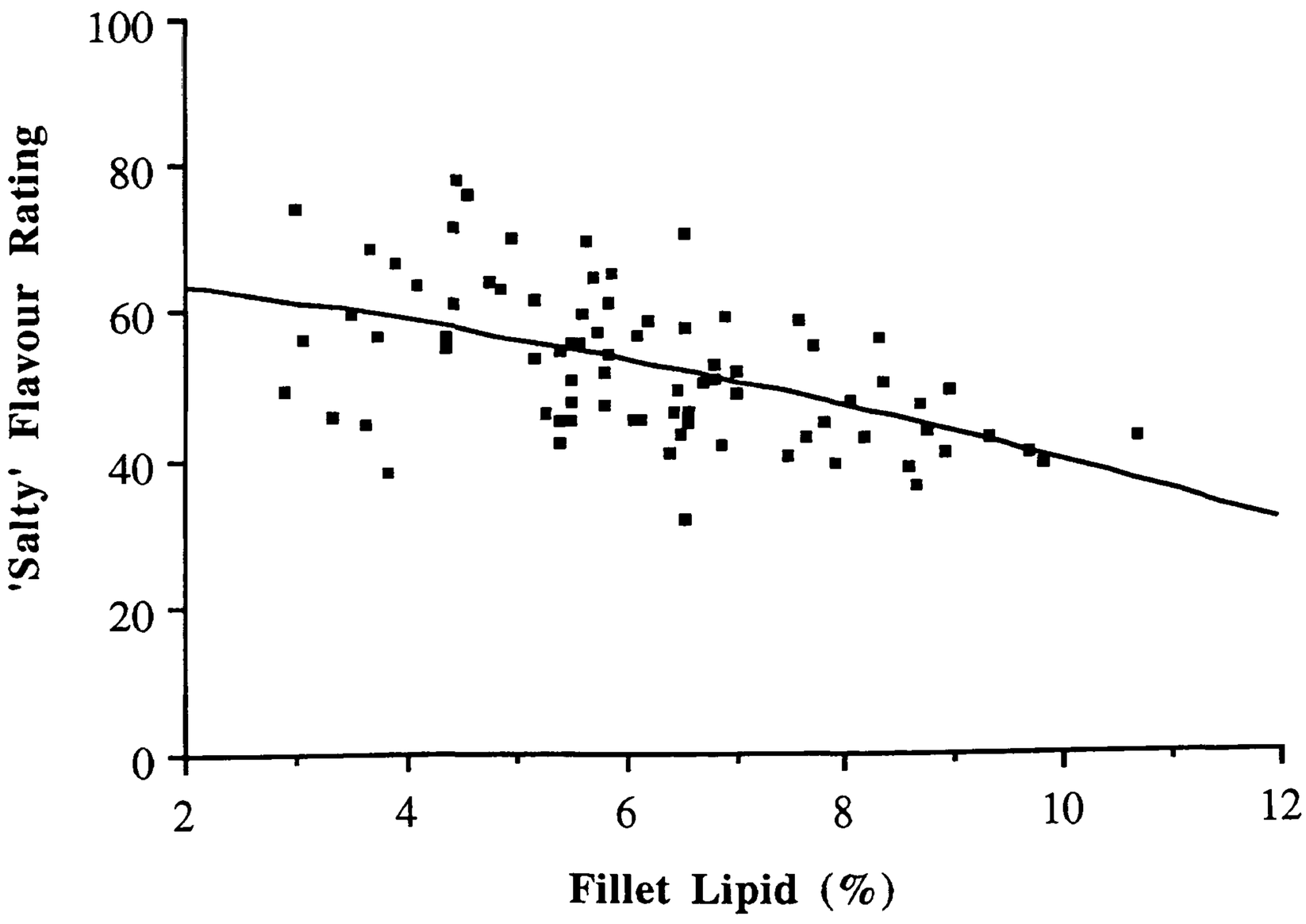

Figure 3.3.17: Relation between *salty* flavour and lipid content.
$y = -0.12x^2 - 1.49x + 66.79$, $r^2 = 0.281$

Figure 3.3.17 shows that the variation of *salty* flavour was greater in the lower lipid contents — below about 5% — but this degree of variation appeared to lessen as the lipid content increased. The *salty* flavour also decreased with increasing lipid, supporting the results found from the analysis of the group means.

iii) Smoky

Smoky was another important flavour attribute. The mean results from each group are shown in table 3.3.17. From this it can be seen that there was no effect of the groups on the *smoky* flavour ($p>0.05$).

Table 3.3.17: Mean ratings for the flavour attribute *smoky*. The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	31.65	32.33	30.08	28.36	1.073	<0.001

Figure 3.3.18 shows a plot of the mean ratings for each fillet against the lipid content. From the figure it can be seen that there was no relationship between lipid content and the rating for the *smoky* flavour ($p>0.05$). The *smoky* flavour appeared to be approximately constant throughout the range of lipid contents.

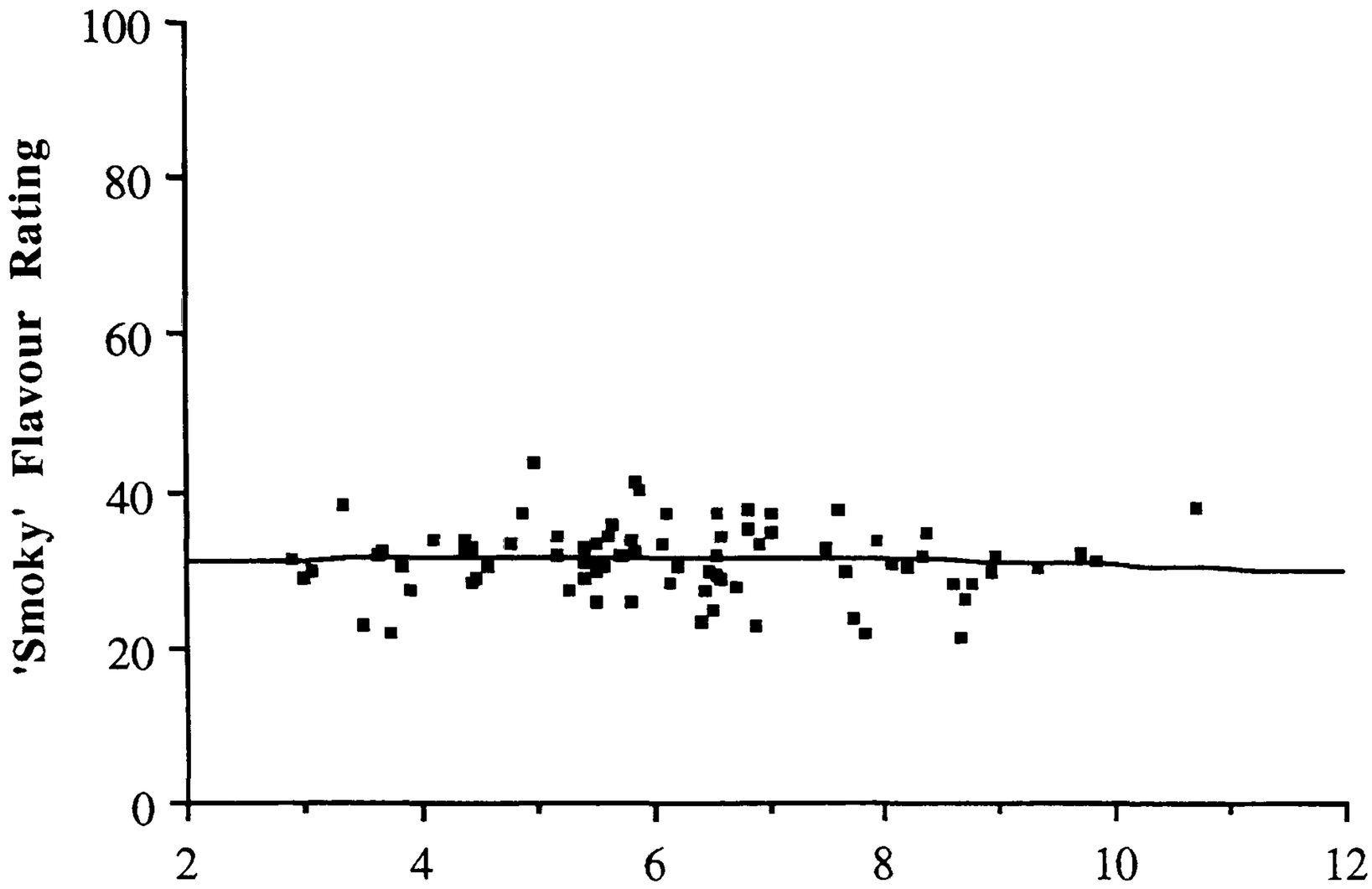

Figure 3.3.18: Relation between *smoky* flavour and lipid content.
$y = -0.05x^2 + 0.60x + 29.67$, $r^2 = 0.002$

iv) Sour

Sour flavour was the next flavour attribute. From table 3.3.18, which shows the mean ratings for each group, it can be seen that there was a significant effect of the groups on the *sour* flavour. However, examination of the means showed that there was no trend with increasing lipid content and the results seemed random.

Table 3.3.18: Mean ratings for the flavour attribute *sour*. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	10.78 [a]	8.38 [b]	10.30 [a]	7.37 [b]	1.097	<0.001

Figure 3.3.19 shows the plot of the mean ratings for *sour* flavour against the fillet lipid content. The correlation coefficient for the plot was $r=0.338$ ($p<0.01$). It can also be seen that the ratings were very low, which indicated that the flavour was of a low intensity, which may have made it hard for some panellists to detect.

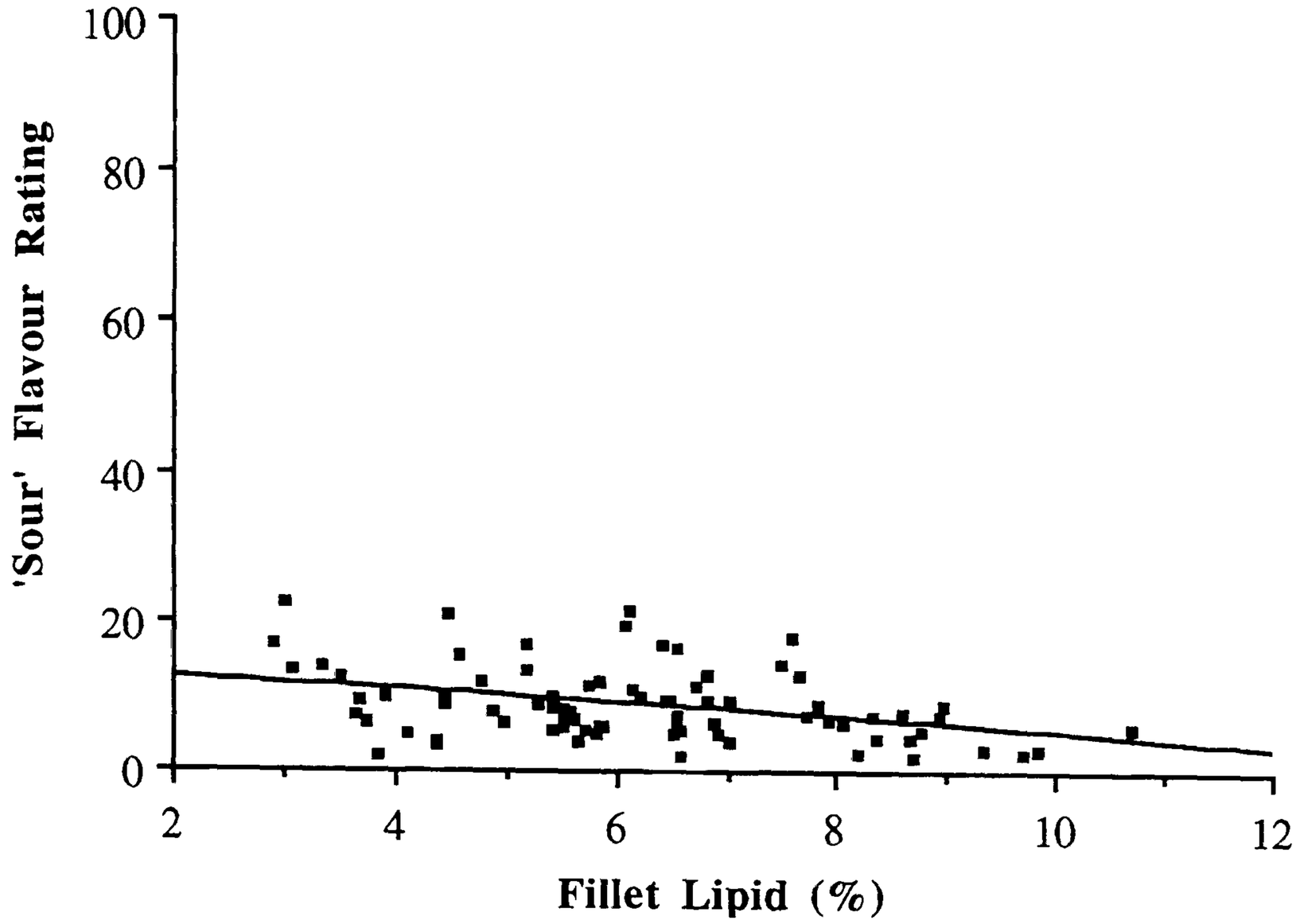

Figure 3.3.19: Relation between *sour* flavour and lipid content.
$y = -0.02x^2 - 0.64x + 13.74$, $r^2 = 0.114$

v) Oily

Oily flavour was another important flavour attribute, as it was presumed that this would be strongly affected by lipid content. Table 3.3.19 shows the mean ratings for each group. There was a significant effect of the groups on *oily* flavour ratings ($p<0.001$). There was no difference between the 2-5% and 5-6% lipid groups, but differences occurred between the 5-6% group and the 6-7% and 7-10% groups. This showed that, as expected, *oily* flavour increased with increasing lipid.

Table 3.3.19: Mean ratings for the flavour attribute *oily*. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	25.84 ^{ab}	25.47 ^{a}	28.57 ^{b}	31.10 ^{c}	1.504	<0.001

The mean *oily* flavour ratings for each fillet were plotted against the fillet lipid content (figure 3.3.20). A regression line fitted to the data and the correlation coefficient of r=0.344 indicated a significant relationship ($p<0.01$).

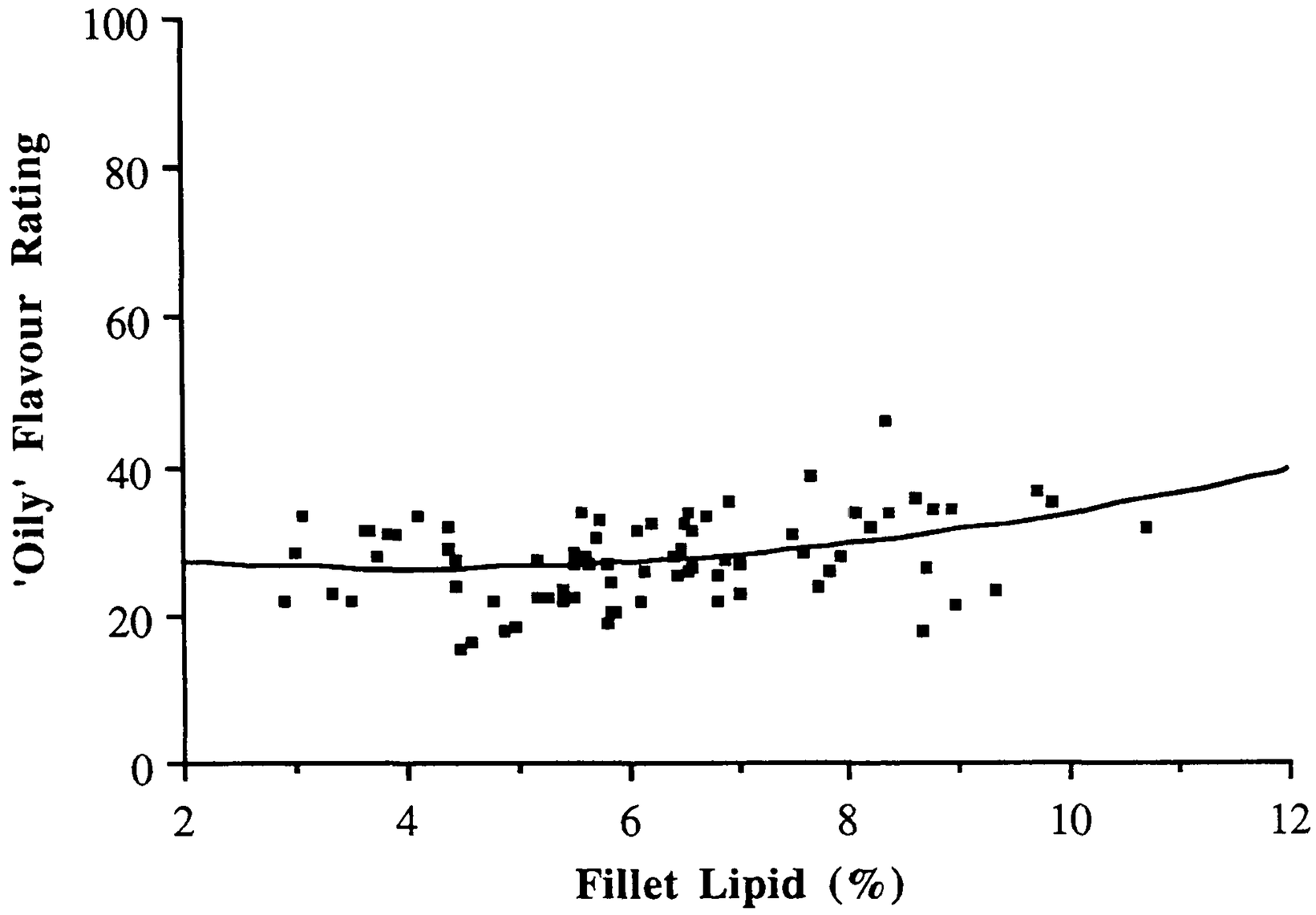

Figure 3.3.20: Relation between *oily* flavour and lipid content.
$y = 0.22x^2 - 1.81x + 29.79$, $r^2 = 0.118$

The *oily* rating was low until approximately 6% lipid when the ratings then started to increase, increasing more rapidly with the higher lipid contents (figure 3.3.20). However, it should be noted that the variation of the ratings in the higher lipid levels was quite large which could cause misinterpretation of the results.

vi) Metallic

The table of means shows that there was no effect of lipid group on this flavour (table 3.3.20). However, the plot of individual fillet ratings against the lipid content (figure 3.3.21) showed a decrease in the ratings with increasing lipid. This relationship had a correlation coefficient of r=0.253, which was significant ($p<0.05$). As with the ratings for the flavour attribute *sour*, it should be noted that the ratings were very low. This meant that the flavour was of a low intensity and thus hard to

detect, which can be seen from the degree of variation especially in the range 4% to 8% lipid.

Table 3.3.20: Mean ratings for the flavour attribute *metallic*. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	7.20	6.78	5.81	5.46	1.283	0.494

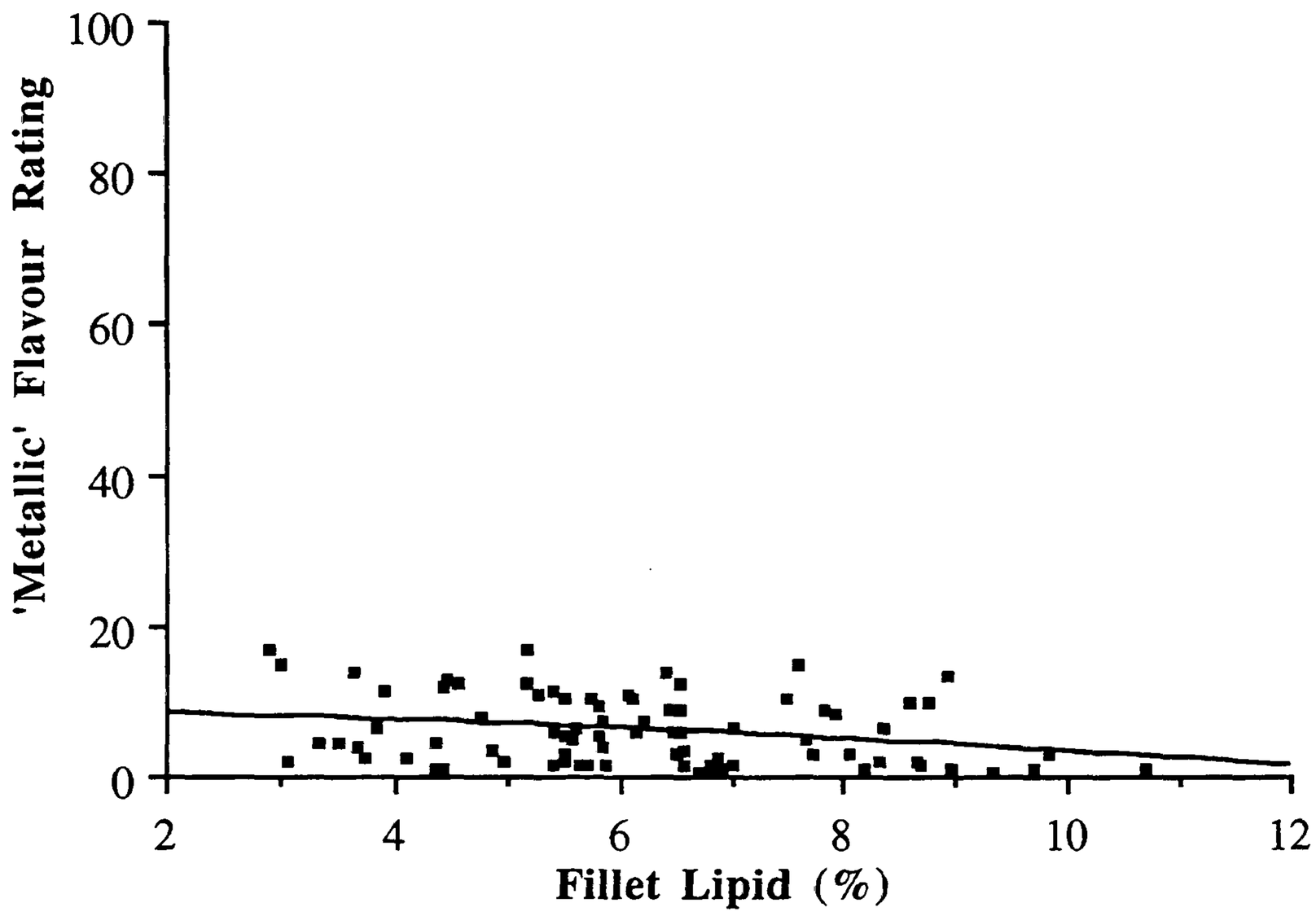

Figure 3.3.21: Relation between *metallic* flavour and lipid content.
$y = -0.03x^2 - 0.25x + 9.04$, $r^2 = 0.064$

3.3.3.5 Overall Rating of Smoked Salmon

i) Flavour

The overall ratings for *flavour* and *liking* were given according to a hedonic scale of 0 (minimum) to 100 (maximum). Table 3.3.21 shows the mean ratings for *overall flavour* for each lipid group. From this it can be seen that lipid group significantly affected the perception of *overall flavour* ($p<0.001$). As the lipid level increased the flavour increased until the 6-7% and 7-10% groups, between which no difference was determined.

Table 3.3.21: Mean ratings for *overall flavour*. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	28.56 [a]	33.75 [b]	37.51 [c]	39.63 [c]	1.517	0.494

The plot of the mean ratings against the lipid content for each individual fillet confirmed this relationship (figure 3.3.22). The correlation coefficient of the regression line fitted to the data was $r=0.559$ ($p<0.001$). The *overall flavour* increased with increasing lipid throughout the range investigated. The maximum flavour rating was not obviously approached in the lipid range used.

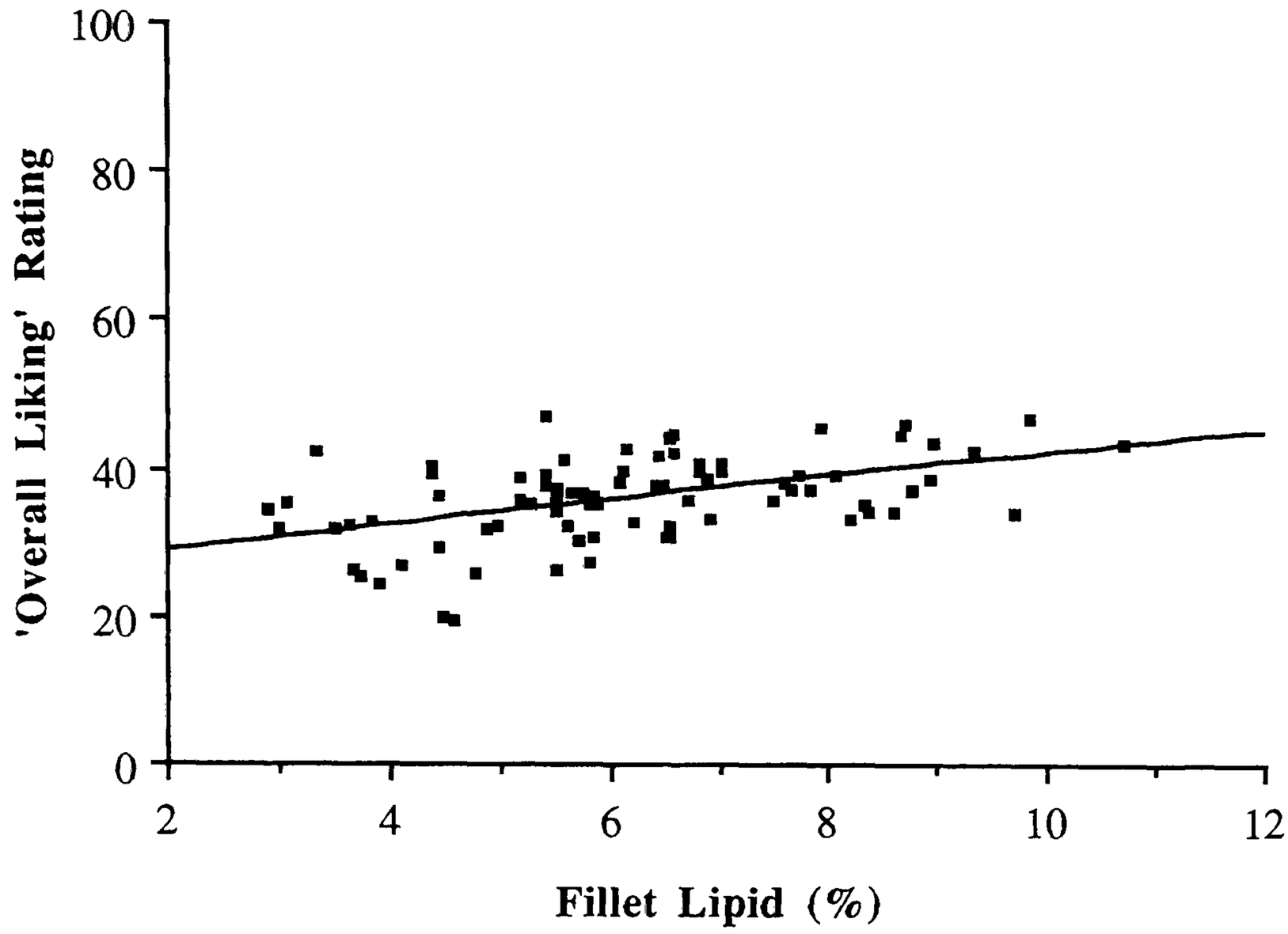

Figure 3.3.22: Relation between *overall flavour* and lipid content.
$y = 0.002x^2 + 2.28x + 20.67$, $r^2 = 0.313$

ii) Liking

Overall liking ratings for each group are shown in table 3.3.22. From this it can be seen that lipid group significantly affected the *overall liking* ($p<0.001$). As with the *overall flavour* ratings, the liking increased over the first two lipid groups and there was no difference between the 6-7% and 7-10% groups which were given the highest ratings.

Table 3.3.22: Mean ratings for *overall liking*. Means with different superscript letters are significantly different ($p<0.05$). The probability in the last column is the probability that the groups did not have an effect on the attribute.

Group	2-5%	5-6%	6-7%	7-10%	s.e.d.	p
Mean	30.81 [a]	35.49 [b]	38.27 [c]	39.45 [c]	1.365	0.494

The individual *liking* ratings were plotted against the lipid content for each fillet (figure 3.3.23). The quadratic regression line was fitted to the data with a correlation coefficient of $r=0.504$ ($p<0.001$). The *overall liking* ratings increased with increasing

lipid throughout the range investigated. This showed that the fish with higher lipid in the range investigated were preferred by this taste panel.

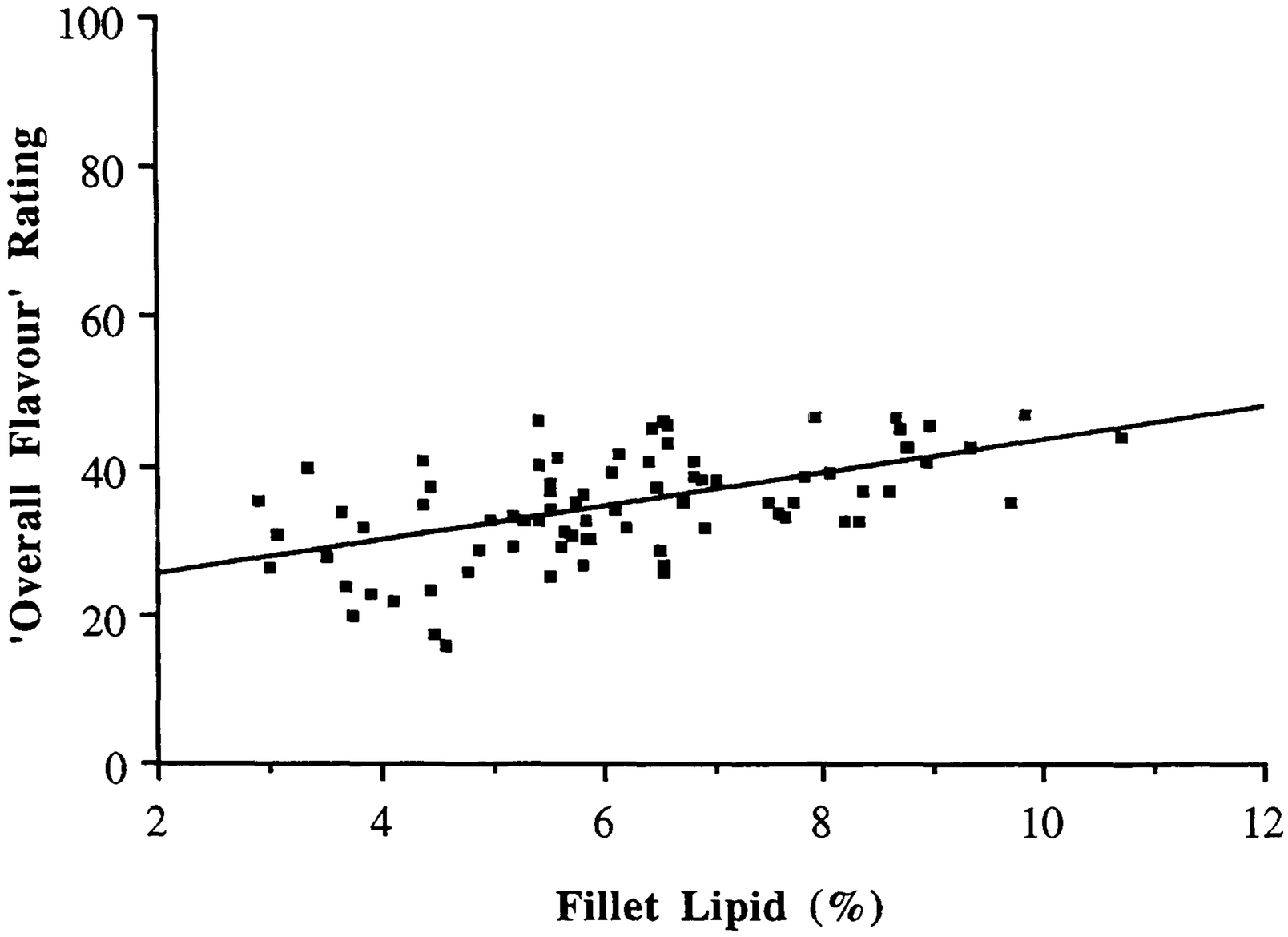

Figure 3.3.23: Relation between *overall liking* and lipid content.
$y = -0.02x^2 + 1.93 + 24.82$, $r^2 = 0.254$

Finally, the individual ratings for overall flavour and liking were plotted on the same graph to see if there was a correlation between flavour and liking. Figure 3.3.24 shows that the regression line which could be fitted to the data had a coefficient of r=0.936. This indicated a significant correlation ($p<0.001$).

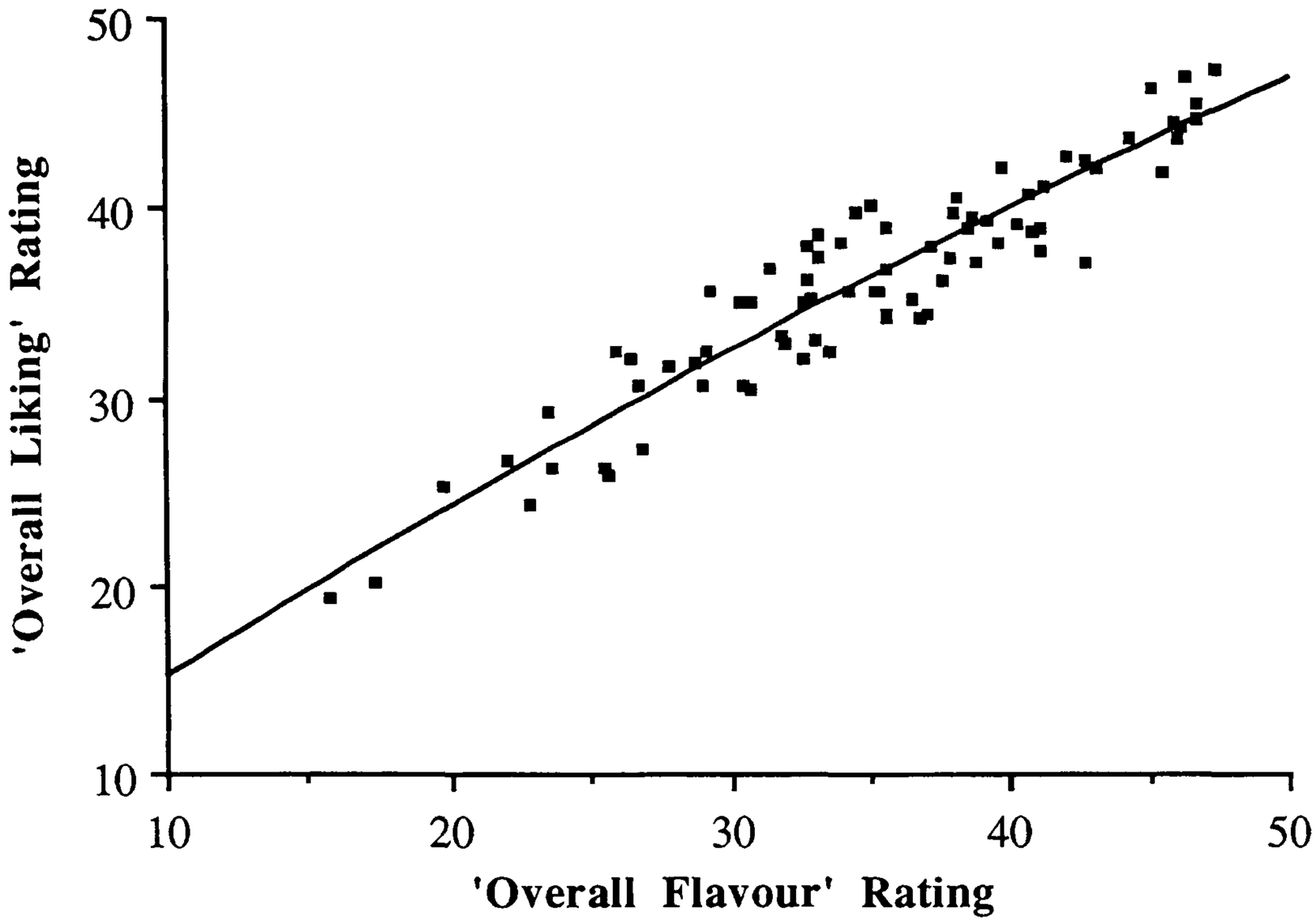

Figure 3.3.24: Relation between *overall liking* and *overall flavour.*

$y = -0.004x^2 + 1.01x + 5.36,\ r^2 = 0.877$

3.3.3.6 Turning Points for Smoked Salmon Attributes

Many of the figures above show that the regression curves which best fit the data have quadratic equations. From the equation of the curves it was possible to calculate the percentage lipid at the turning point, where the lines reached a maximum or minimum, by using differentiation. The results of this are shown in table 3.3.23.

Table 3.3.23 Turning points of the texture and flavour ratings.

Attribute	Lipid Level (%)	Max. or Min.	Significance Level of Curve
Firm Texture on Cut	9.1	Min.	<0.001
Clean-cut Texture on Cut	8.3	Max.	<0.001
Slimy Texture on Bite	10.4	Max.	<0.001
Oily Texture on Bite	8.4	Max.	<0.001
Firm Texture on Bite	9.4	Min.	<0.001
Jellified on Chew	18.5	Max.	<0.01
Moist Texture on Chew	10.7	Max.	<0.001
Firm Texture on Chew	92.6	Min.	<0.01
Dissolubility Texture on Chew	18.2	Max.	<0.01
Cohesive Texture on Chew	-	-	ns
Chewy Texture on Chew	9.3	Min.	<0.01
Fishy Flavour	8.6	Max.	<0.01
Salty Flavour	-6.1	Max.	<0.001
Smoky Flavour	-	-	ns
Sour Flavour	-	-	ns
Oily Flavour	4.1	Min.	<0.01
Metallic Flavour	-4.0	Max.	<0.05
Overall Flavour	746.0	Max.	<0.001
Overall Liking	56.3	Max.	<0.001

From the table it can be seen that many of the attributes reached a maximum or minimum rating in the lipid range investigated. An important lipid level for texture and flavour appeared to be in the range of 8 to 10%. In this range eight of the twenty attributes had turning points. However, it should be noted that the turning points for

overall flavour and *overall liking* lay well above the range investigated. In these two cases the quadratic term is very small and the relationship with lipid content is almost linear.

3.3.4 Cooked Salmon

The taste panel results for the fresh fillets did not show as great a dependence on the flesh lipid content as those for the smoked fillets did. They are therefore not described in as much depth as the results for the smoked fillets. Table 3.3.24 and table 3.3.25 respectively show the mean ratings for the lipid groups for each of the texture and flavour attributes given by the panel.

The mean fillet ratings were plotted against the fillet lipid content for each of the attributes. Regression lines which best fitted the data were plotted on the graphs and the correlation coefficients calculated. The graphs showing significant relationships between lipid and the attribute ratings are shown and discussed below. The remaining attributes were found to be unaffected by the level of lipid in the flesh.

Table 3.3.24: Mean group ratings for each of the texture attributes. The probability quoted in the last column is the probability that the lipid groups have no effect on the attributes ratings (ANOVA). Means in the same row with different superscript letters are significantly different (Fisher PLSD $p<0.05$).

Attribute	2-5% Lipid	5-6% Lipid	6-7% Lipid	7-10% Lipid	Signif.
On Cutting					
Firmness	25 (1.3)	24 (1.2)	24 (0.9)	23 (1.2)	ns
Disintegration	41 (1.2)	42 (1.6)	41 (1.5)	44 (2.1)	ns
On First Bite					
Slimy	12 (1.0)	12 (0.9)	12 (1.0)	12 (1.0)	ns
Firm	21 (1.6)	21 (1.1)	21 (1.3)	19 (1.0)	ns
Moist	38 (1.7) ^{a}	41 (1.1) ^{ab}	44 (1.2) ^{b}	42 (1.6) ^{b}	$p< 0.05$
Sticky	24 (1.2)	24 (1.1)	26 (0.8)	26 (0.9)	ns
Gelatinous	3 (0.5)	4 (0.5)	5 (0.5)	5 (0.5)	ns
Chewy	22 (0.8) ^{ab}	24 (0.7) ^{a}	23 (1.1) ^{ab}	21 (0.7) ^{b}	$p< 0.1$
Fibrous	15 (0.7) ^{a}	16 (0.3) ^{b}	16 (0.4) ^{b}	16 (0.5) ^{b}	$p<0.1$
Cohesive	35 (1.2) ^{a}	38 (0.8) ^{b}	37 (0.7) ^{ab}	34 (1.0) ^{a}	$p< 0.05$
Dissolubility	28 (1.3)	26 (0.9)	28 (1.3)	26(1.2)	ns

Table 3.3.25: Mean group ratings for each of the flavour attributes. The probability quoted in the last column is the probability that the lipid groups have no effect on the attributes ratings (ANOVA). Means in the same row with different superscript letters are significantly different (Fisher PLSD $p<0.05$).

Attribute	2-5% Lipid	5-6% Lipid	6-7% Lipid	7-10% Lipid	Signif.
Flavour					
Fishy	33 (1.1)	34 (1.2)	36 (1.2)	34 (1.0)	ns
Bitter	9 (0.9)	9 (0.8)	9 (0.9)	11 (1.1)	ns
Creamy	16 (1.0)	17 (1.0)	16 (1.2)	16 (1.0)	ns
Seaweed	12 (1.0) [a]	12 (0.8) [ab]	14 (1.0) [ab]	14 (1.1) [b]	ns
Oily	13 (0.7)	14 (0.7)	14 (0.9)	14 (0.8)	ns
Sour	7 (0.6)	6 (0.5)	7 (0.6)	7 (0.7)	ns
Sweet	6 (0.7)	7 (0.8)	7 (0.9)	6 (0.6)	ns
Metallic	4 (0.6)	3 (0.4)	4 (0.6)	4 (0.4)	ns
Earthy	17 (1.0) [ab]	18 (0.9) [a]	16 (1.2) [ab]	16 (0.9) [b]	ns
Stale	11 (1.0)	10 (1.0)	10 (0.9)	10 (0.8)	ns
Salty	3 (0.4)	3 (0.4)	3 (0.5)	2 (0.3)	ns
Overall					
Flavour	37 (1.6)	38 (1.2)	37 (1.3)	38 (1.4)	ns
Liking	38 (1.5)	40 (1.1)	40 (1.1)	39 (1.3)	ns

3.3.4.1 Texture of Cooked Salmon

i) Firm on First Bite

Figure 3.3.25 shows the results for the attribute *firm* texture on first bite and the relation between lipid and the texture rating. The correlation coefficient was calculated as r=0.298 ($p<0.05$). For the lower lipid levels investigated, the *firm* texture rating was approximately constant and then started to drop when the lipid rose above about 6%. It can also be seen that there tended to be more variation in the ratings in the fillets with lower lipid contents. This might have caused some confusion with interpreting the results. As the comparison of the lipid groups showed no relation between lipid group and this texture attribute, and the correlation coefficient of the regression line is low, although significant, the reliability of this analysis may be questioned.

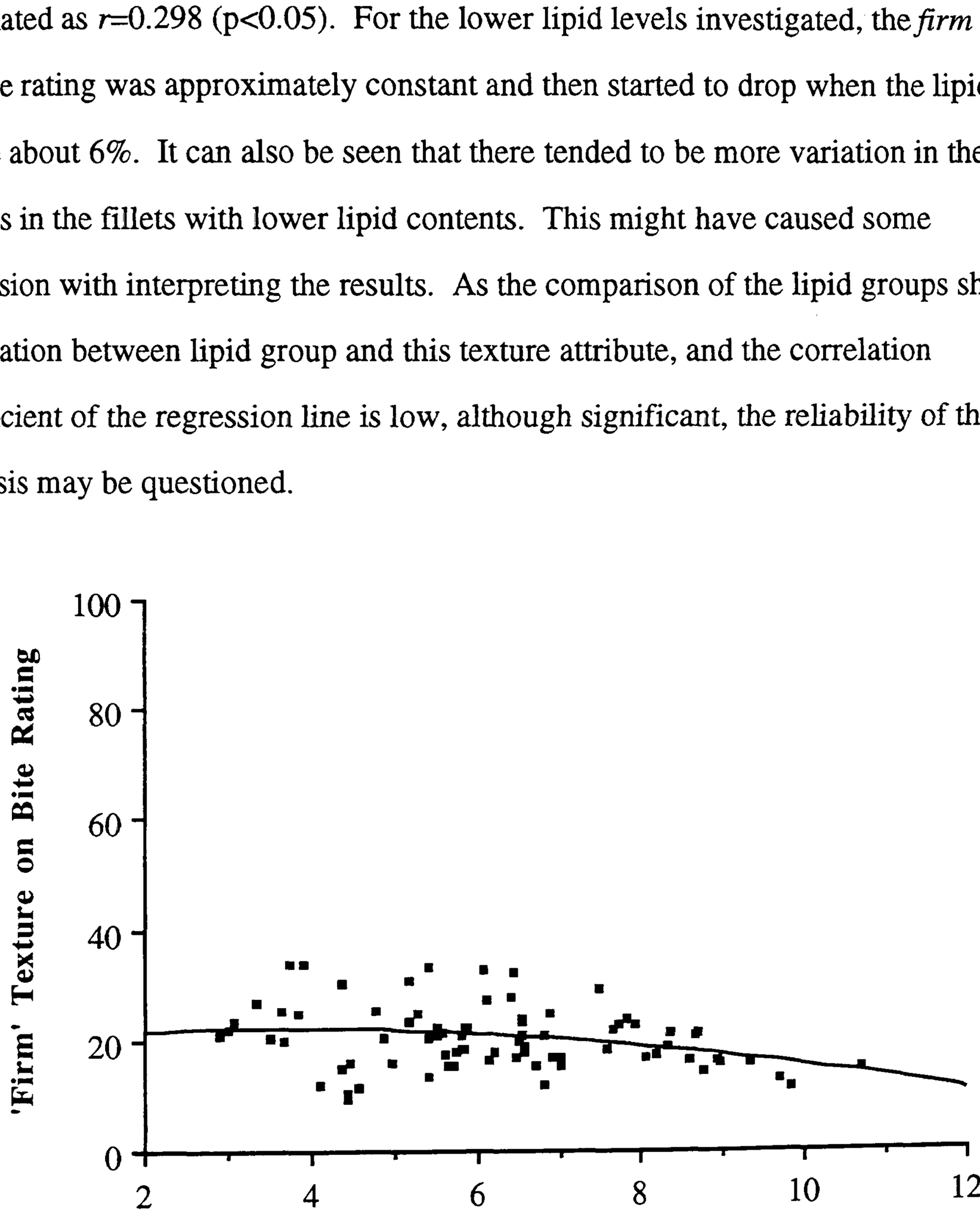

Figure 3.3.25: Relation between *firm* texture on first bite and lipid content.
$y = -0.16x^2 + 1.19x + 19.72$, $r^2 = 0.089$

ii) Moist on First Bite

The *moist* texture attribute has been shown to be significantly affected by lipid group. This was confirmed by the results of the plot of the individual mean ratings against the individual lipid levels (figure 3.3.26). The correlation coefficient for the regression line was r=0.290 ($p<0.05$).

From figure 3.3.26 it can be seen that the *moist* texture rating increased with increasing lipid over the range 2% to about 6%. The rating remained approximately constant over the range 6-8% and then the regression line dropped. However, there was a large degree of variation throughout the range of lipid contents, which may result in the reliability of the correlation being questioned.

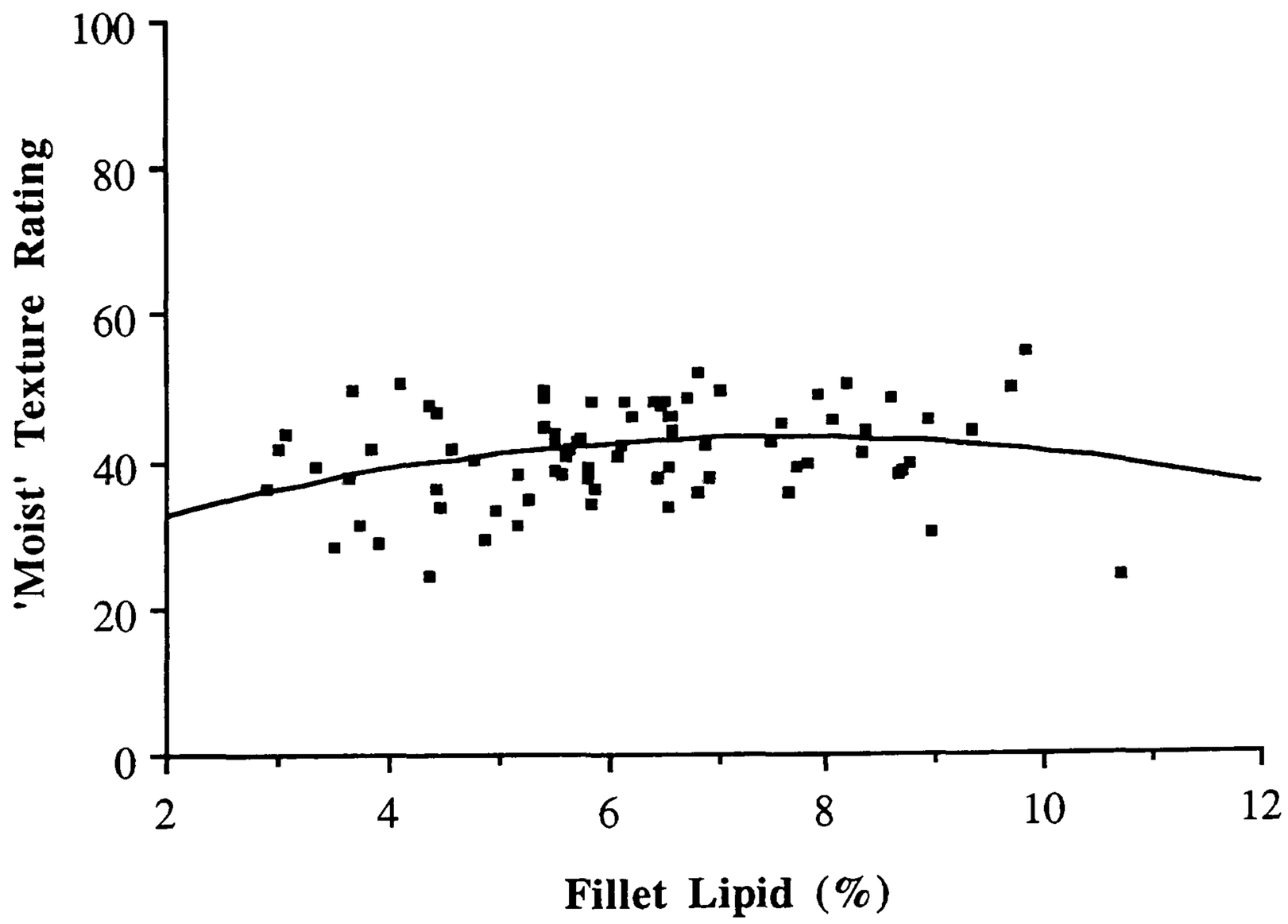

Figure 3.3.26: Relation between *moist* texture and lipid content.
$y = -0.34x^2 + 5.18 + 23.51$, $r^2 = 0.084$

iii) Chewy on First Bite

The *chewy* texture was also significantly affected by lipid group and was shown to be affected by individual lipid. Figure 3.3.27 shows the plot of the individual mean ratings against the individual lipid. The correlation coefficient of r=0.253 was significant ($p<0.05$).

The results from the plot confirm those shown from the analysis of the lipid group means. As lipid content increased over the low lipid levels, *chewiness* increased. From approximately 5% to 7% lipid the ratings were constant and then started to drop again in the higher lipid fillets.

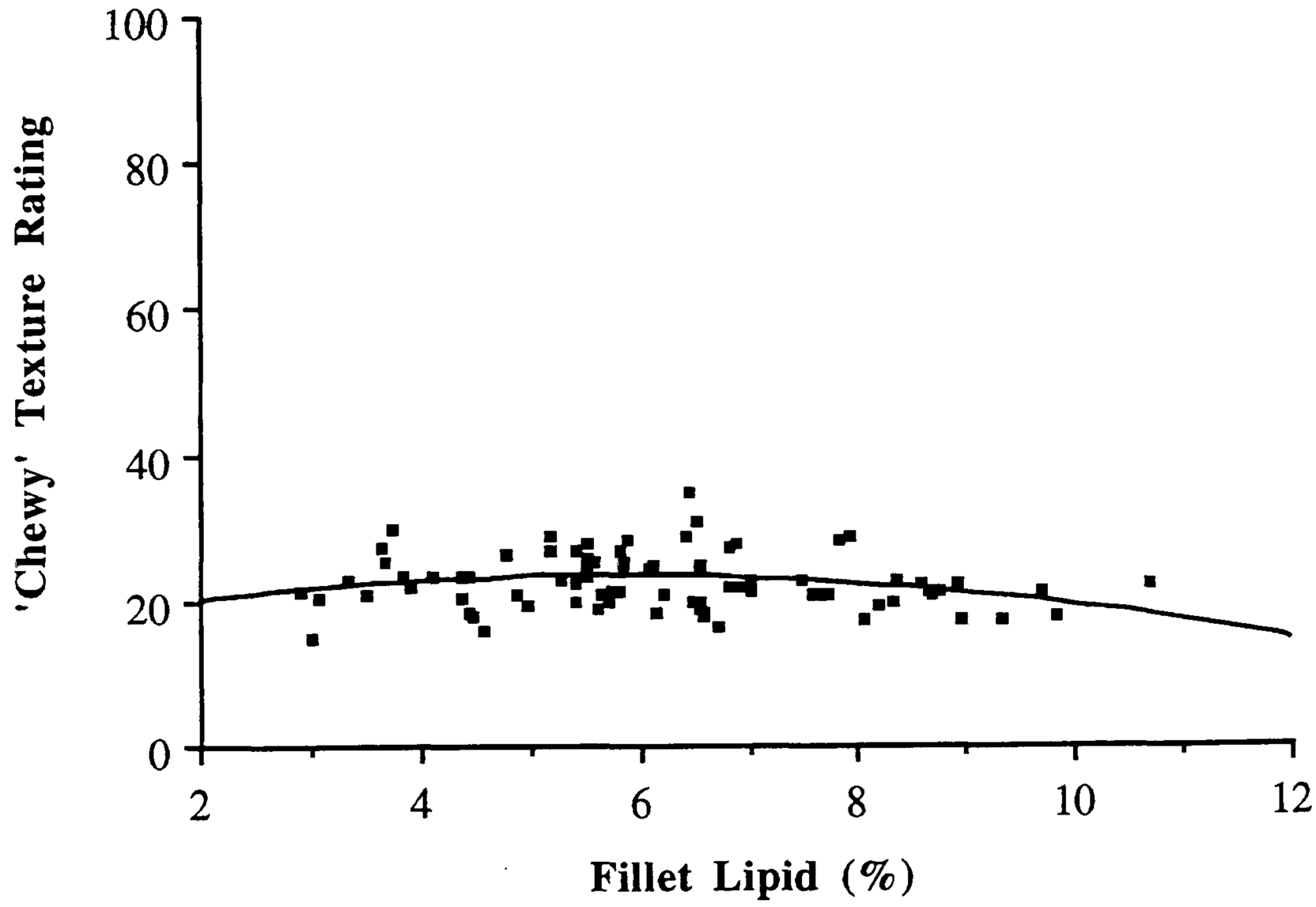

Figure 3.3.27: Relation between *chewy* texture and lipid content.

$y = -0.23x^2 + 2.64x + 15.63$, $r^2 = 0.064$

iv) Fibrous on First Bite

Figure 3.3.28 shows the plot of the individual mean ratings for the attribute *fibrous* on chewing against the individual lipid values. The correlation coefficient of the regression line was calculated as r=0.243, which was significant ($p<0.05$).

As was also shown by the analysis of the mean group ratings, the *fibrous* rating rose with increasing lipid over the range 2% to about 6% (figure 3.3.28). The rating then remained constant until about 9% when it started to drop slightly.

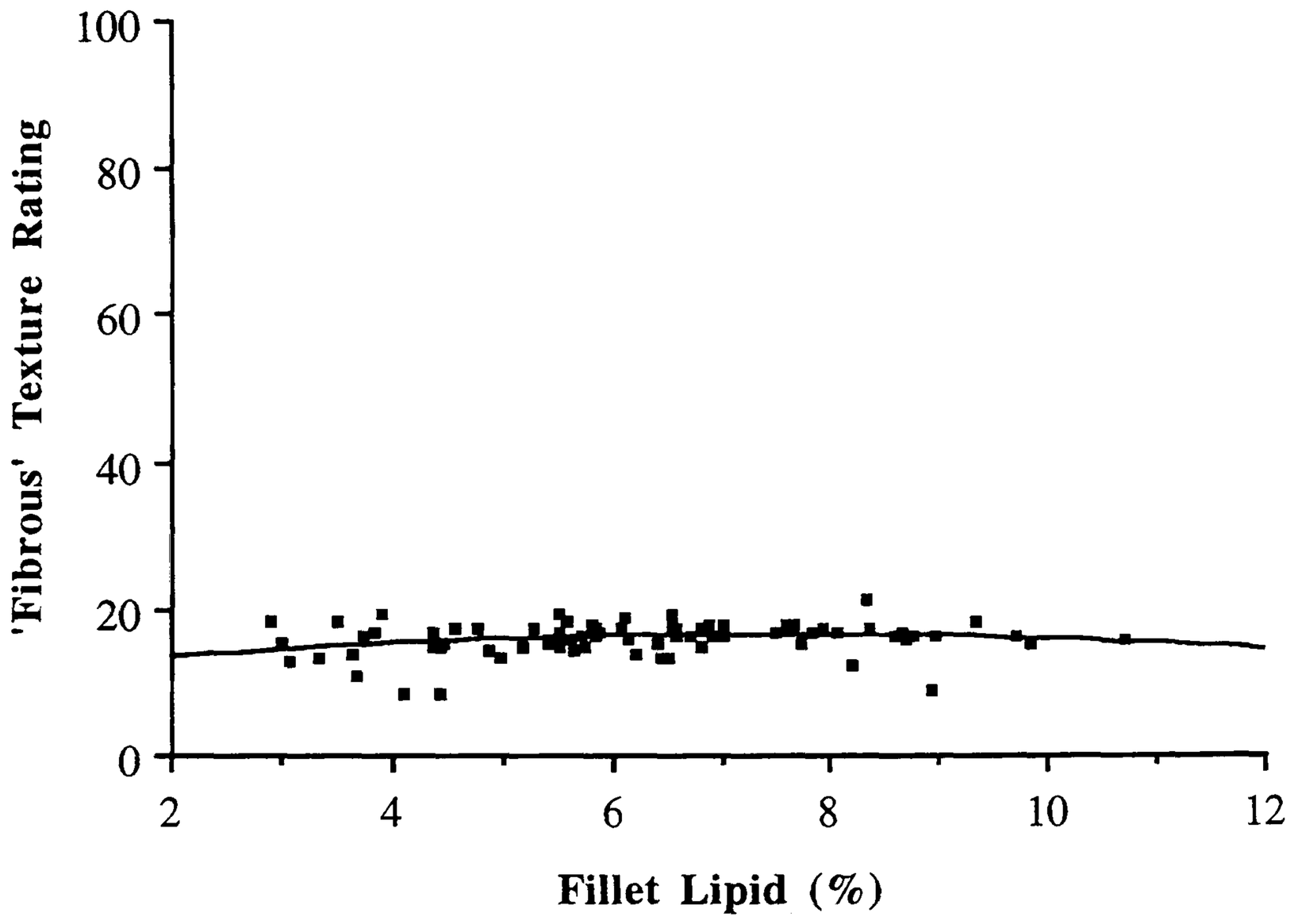

Figure 3.3.28: Relation between *fibrous* texture and lipid content.
$y = -0.10x^2 + 1.52x = 10.74$, $r^2 = 0.059$

v) Cohesive on First Bite

The *cohesive* texture of the sample on chewing had been shown to be significantly affected by the lipid groups. This was confirmed by the plot of the individual ratings (figure 3.3.29). The regression line fitted to the data had a correlation coefficient of r=0.245, which was significant ($p<0.05$).

From figure 3.3.29 it can be seen that there was a slight increase in ratings over the range of 2% to 5%. Above 8% lipid the ratings dropped rapidly over the range investigated.

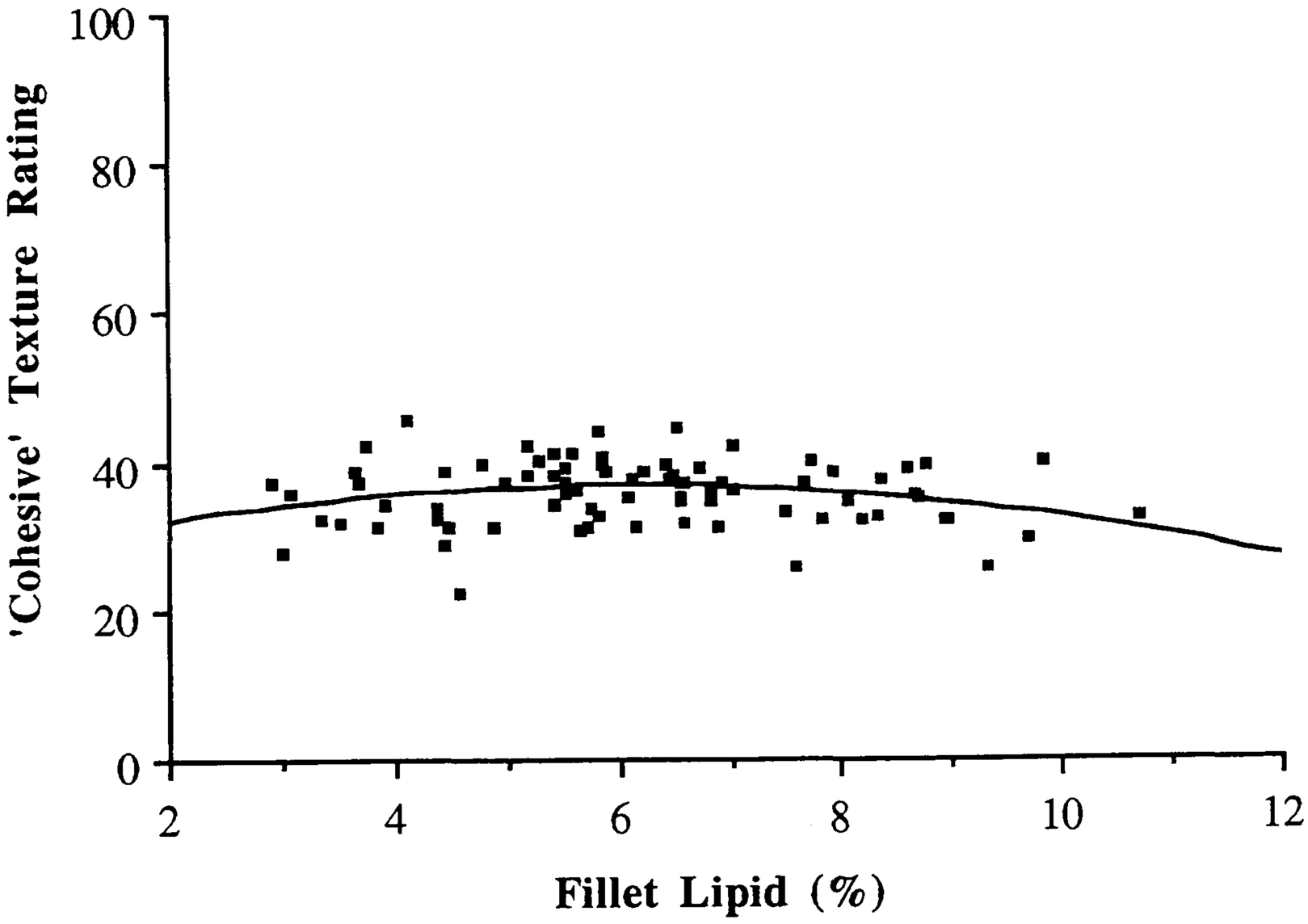

Figure 3.3.29: Relation between *cohesive* texture and lipid content
$y = -0.28x^2 + 3.42 + 26.21$, $r^2 = 0.060$

3.3.4.2 Flavour of Cooked Salmon

Of the eleven flavour attributes detected only four were affected by the lipid group.

i) Bitter

Bitter flavour ratings are shown in figure 3.3.30. The regression line fitted to the data had a correlation coefficient of r=0.214, which was significant ($p<0.05$). From figure 3.3.30 it can be seen that over the range of lipid investigated the rating for *bitter* flavour remained approximately constant from 2% to 6%. Above this the ratings rose with increasing lipid.

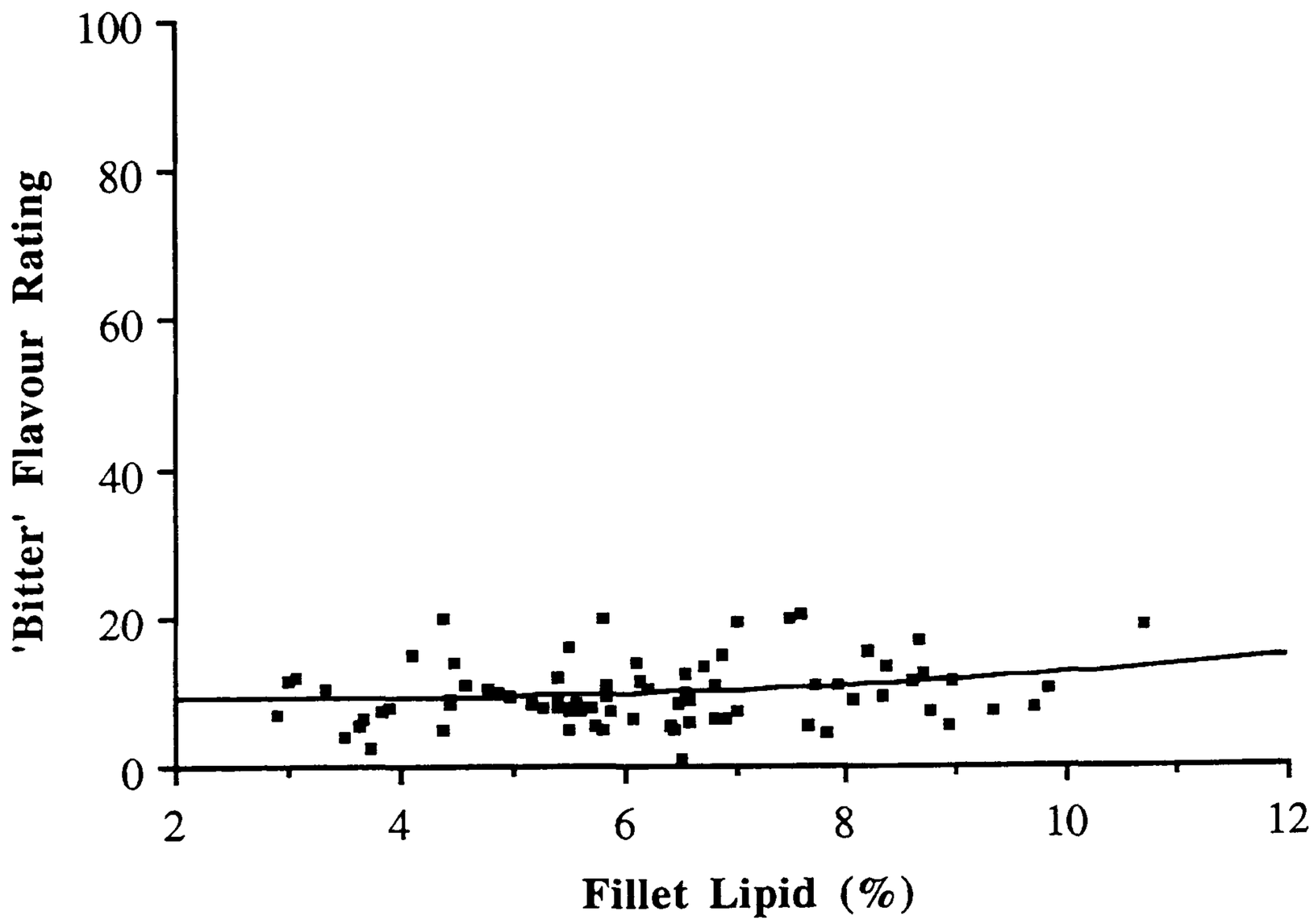

Figure 3.3.30: Relation between *bitter* flavour and lipid content.

$y = 0.06x^2 - 0.30x + 9.10$, $r^2 = 0.046$

ii) Seaweed

Seaweed flavour was rated fairly low. The plot of the individual ratings (figure 3.3.31) allowed a regression line with a correlation coefficient of r=0.205 to be plotted. This indicated a non-significant ($p<0.1$) tendency for lipid content to affect the flavour rating.

From figure 3.3.31 it can be seen that the ratings increased over the range 2% to 6% and then remained fairly constant. This confirmed the results observed for the lipid group means analysis.

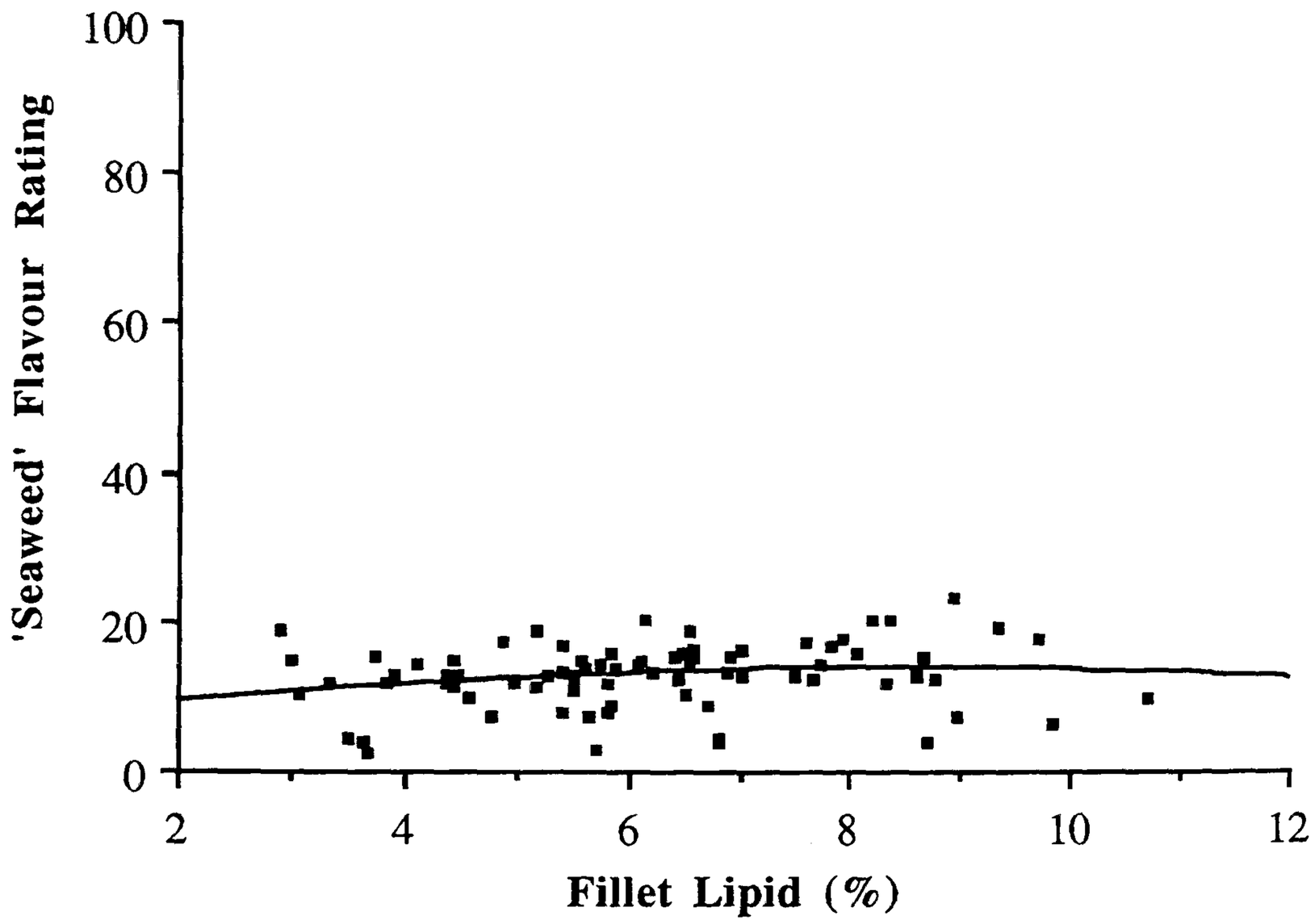

Figure 3.3.31: Relation between *seaweed* flavour and lipid content.
$y = 0.11x^2 + 1.81x + 6.11$, $r^2 = 0.042$

iii) Oily

Mean group rating analysis showed that *oily* flavour was not affected by the lipid groups, which was unexpected (table 3.3.25). However, figure 3.3.32 shows that there was a relationship between lipid and the flavour rating. The regression line fitted to the data had a correlation coefficient of r=0.354, which was significant ($p<0.01$).

From figure 3.3.32 it can be seen that throughout the range of lipid investigated the *oily* flavour rating increased. This was the result expected, although the effect was very small owing to the high degree of variation shown — the degree of variation probably masked the effect of the lipid groups on the attribute leading to the non-significant result shown in table 3.3.25.

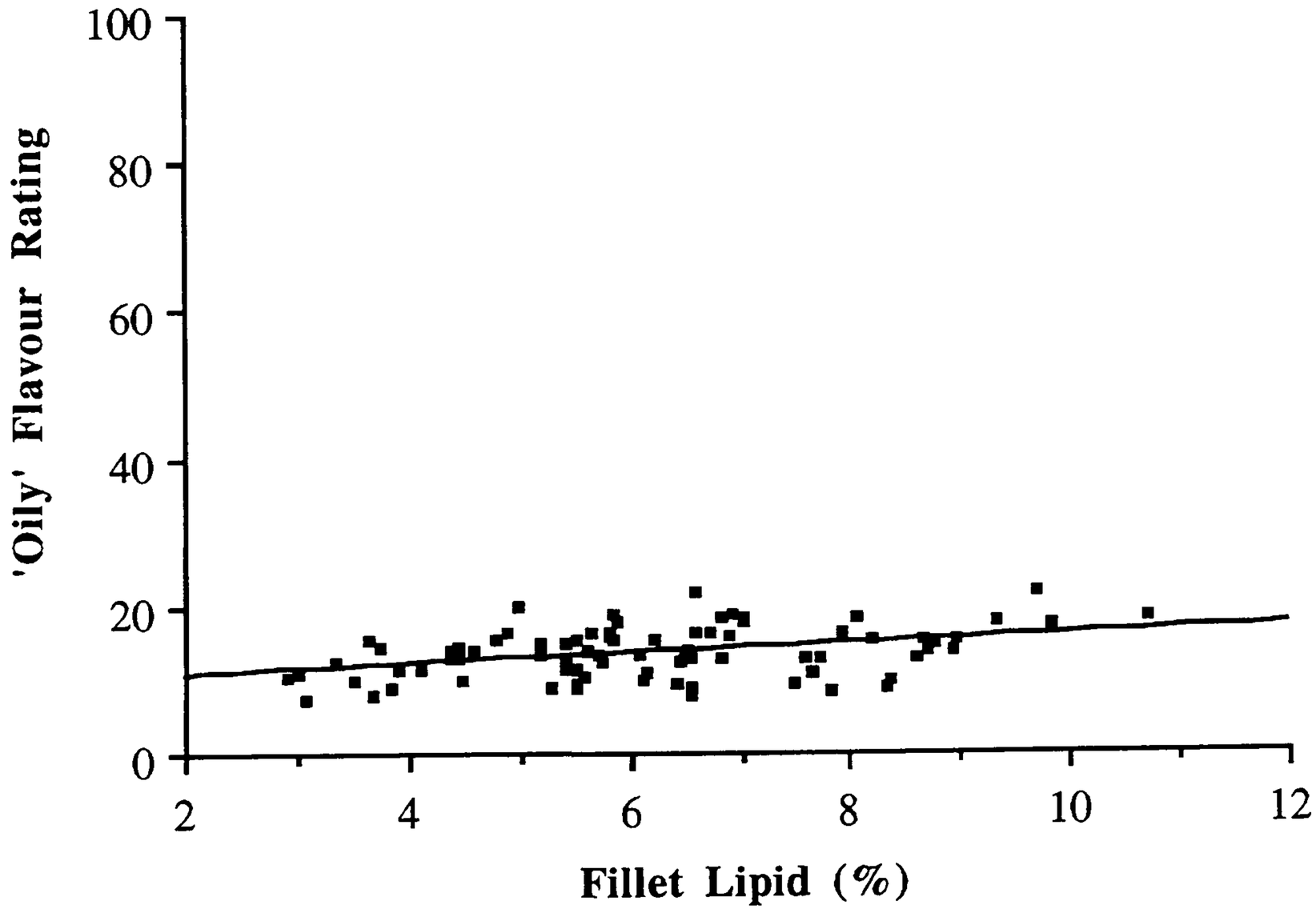

Figure 3.3.32: Relation between *oily* flavour and lipid content.
$y = -0.01x^2 + 0.86x + 8.97$, $r^2 = 0.125$

iv) Sour

Sour flavour in the fillets was rated very low. However, figure 3.3.33 shows that a relation between the rating and lipid content could be determined. The correlation coefficient of the regression line fitted to the data was r=0.259 ($p<0.05$).

From figure 3.3.33 it can be seen that the flavour was rated very low in the range 2% to 6% lipid. The ratings then started to increase slightly towards the end of the range investigated. This was very similar to the results shown for *bitter* flavour.

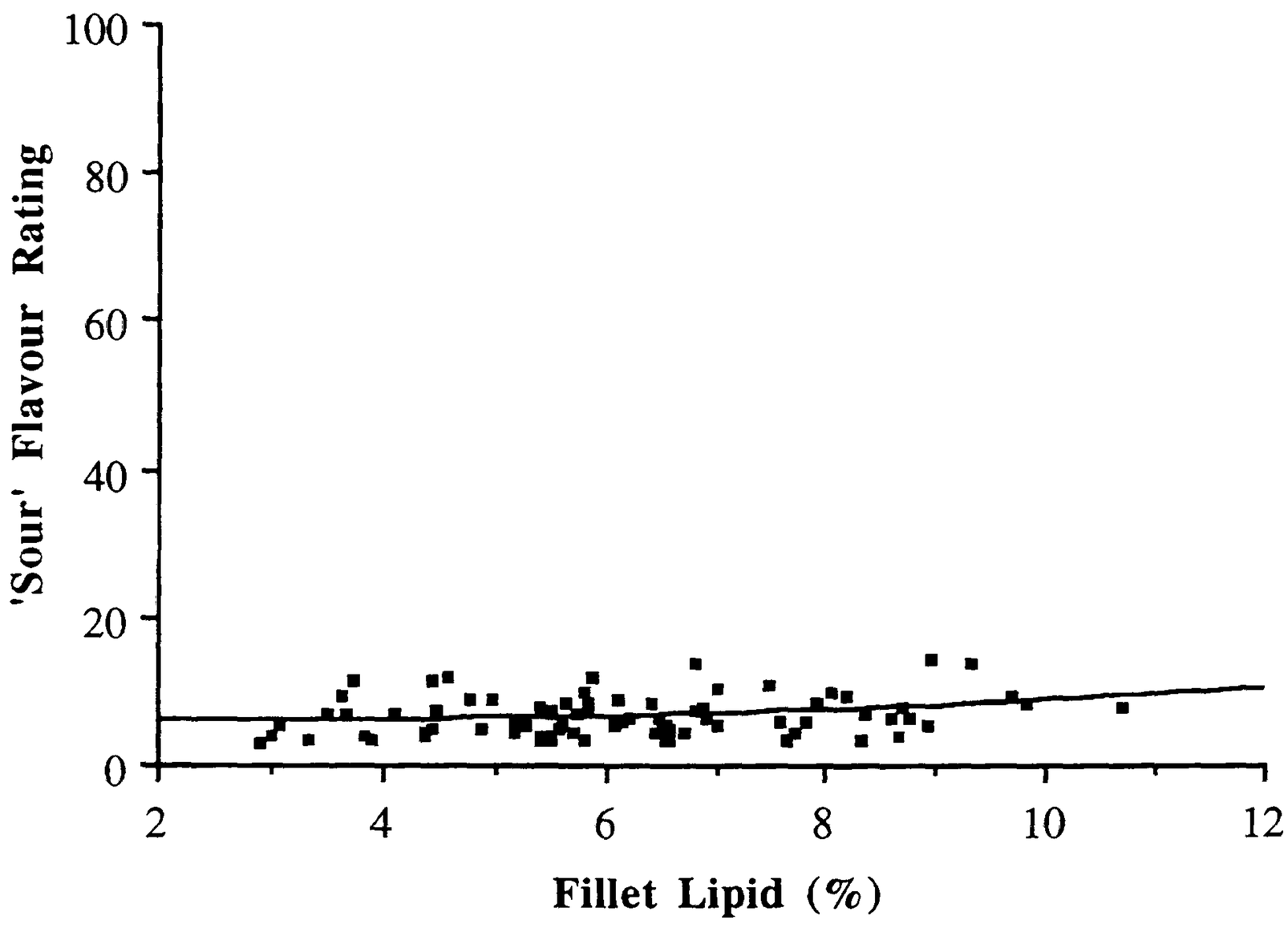

Figure 3.3.33: Relation between *sour* flavour and lipid content.
$y = 0.06x^2 - 0.33x + 6.53$, $r^2 = 0.067$

v) Earthy

Earthy flavour was the second highest rated flavour. Although it was significantly affected by the lipid group, figure 3.3.34 shows that there was no significant correlation between this flavour attribute and individual lipid content ($p>0.05$)

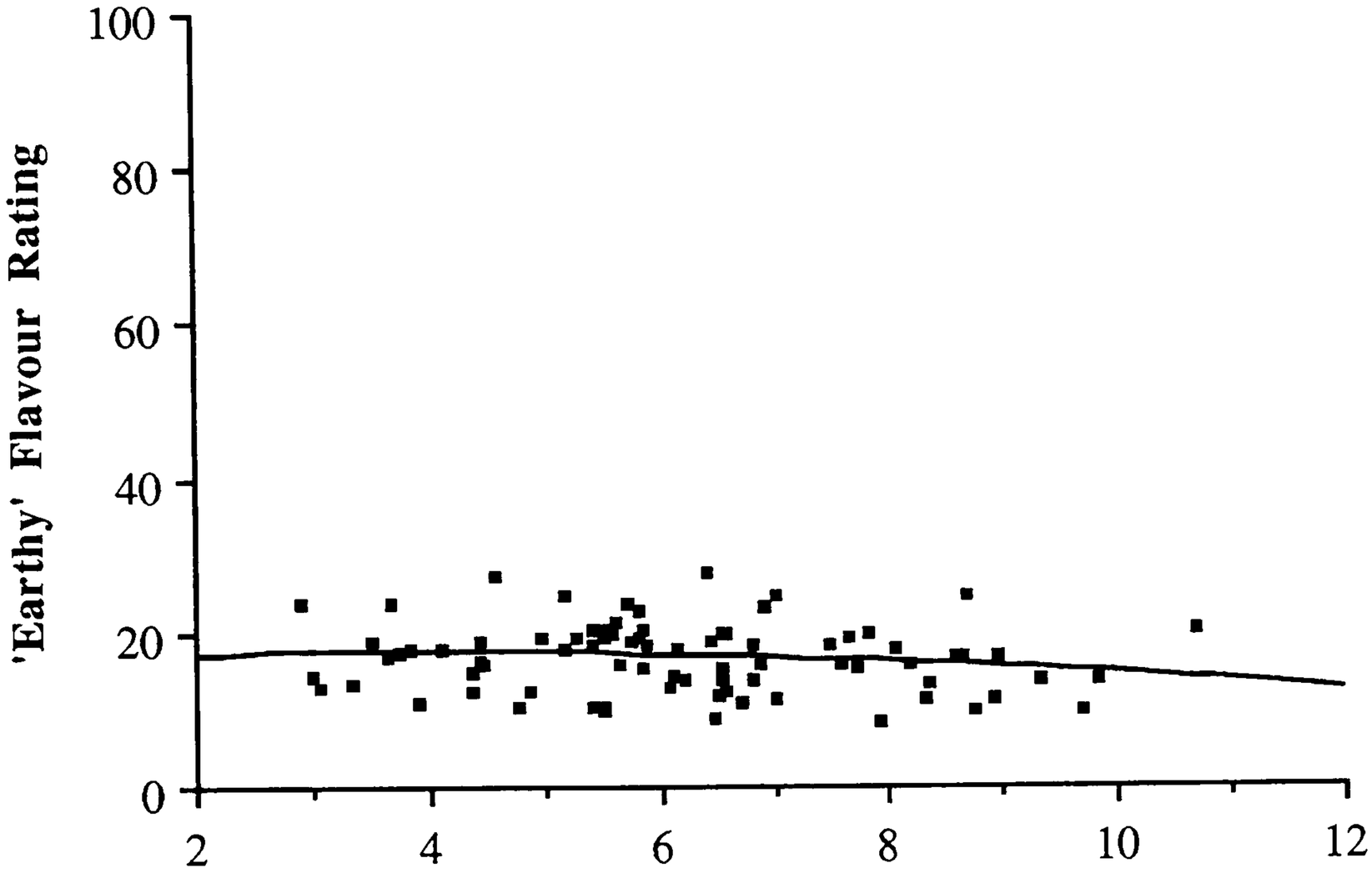

Figure 3.3.34: Relation between *earthy* flavour and lipid content
$y = -0.08x^2 + 0.64x + 16.05$, $r^2 = 0.024$

3.3.4.3 Overall Rating of Cooked Salmon

The overall ratings for *flavour* and *liking*, rated on a hedonic scale of 0 (minimum) to 100 (maximum), were not found to be affected by the lipid groups. This was supported by the results of the regression analyses.

i) Flavour

Figure 3.3.35 shows the plot of *overall flavour* ratings against lipid content. *Overall flavour* was almost completely independent of the lipid content.

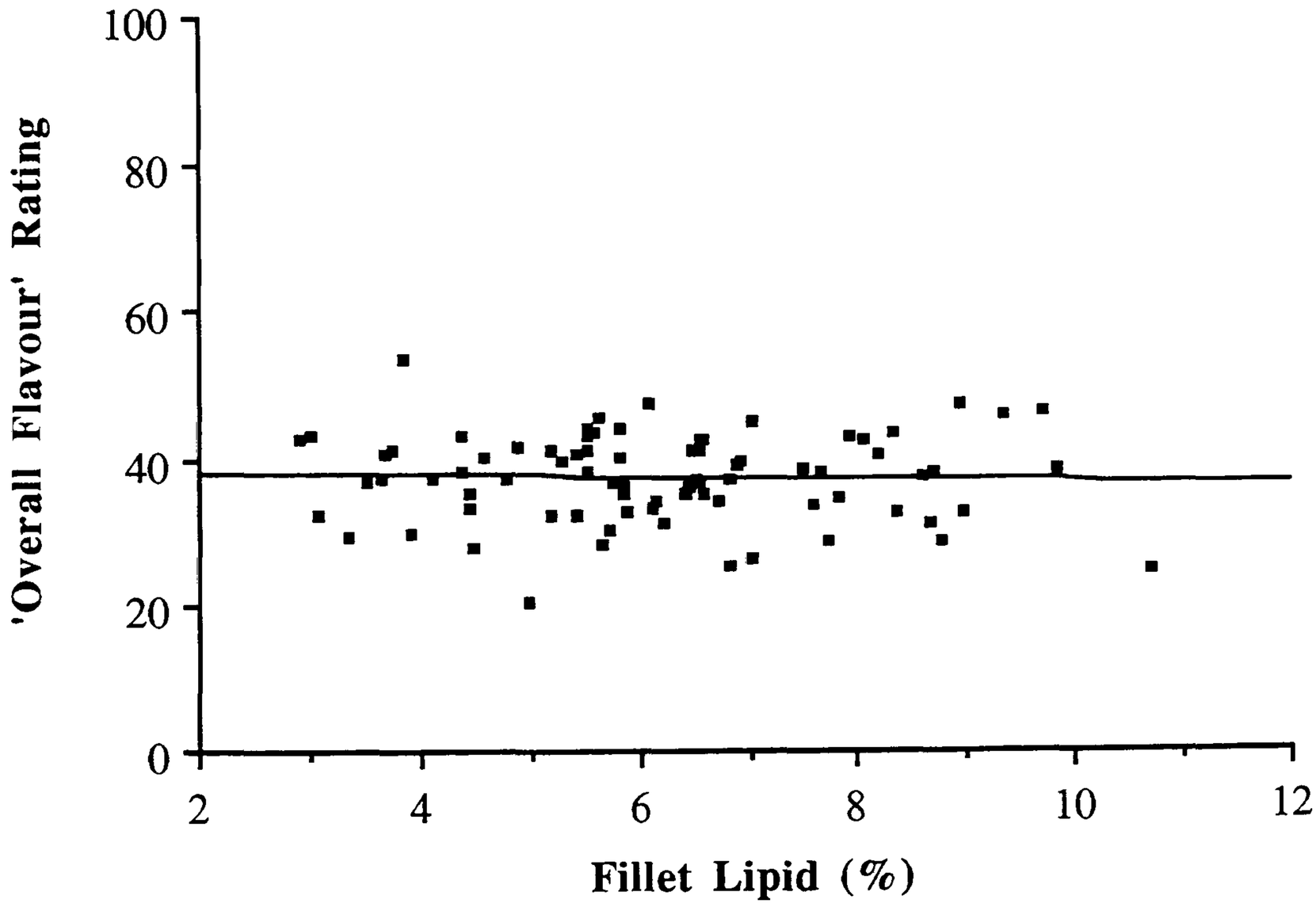

Figure 3.3.35: Relation between *overall flavour* and lipid content.
$y = -0.002x^2 - 0.07x + 38.00$, $r^2 = 0.001$

ii) Liking

Figure 3.3.36 shows the plot of *overall liking* against lipid content. Again, the *overall liking* rating appeared to be almost completely independent of the lipid content. The variation in the ratings for both *flavour* and *liking* was quite high, which may either have masked an effect, or may indicate that another unknown factor affected the *overall flavour* and *liking* ratings.

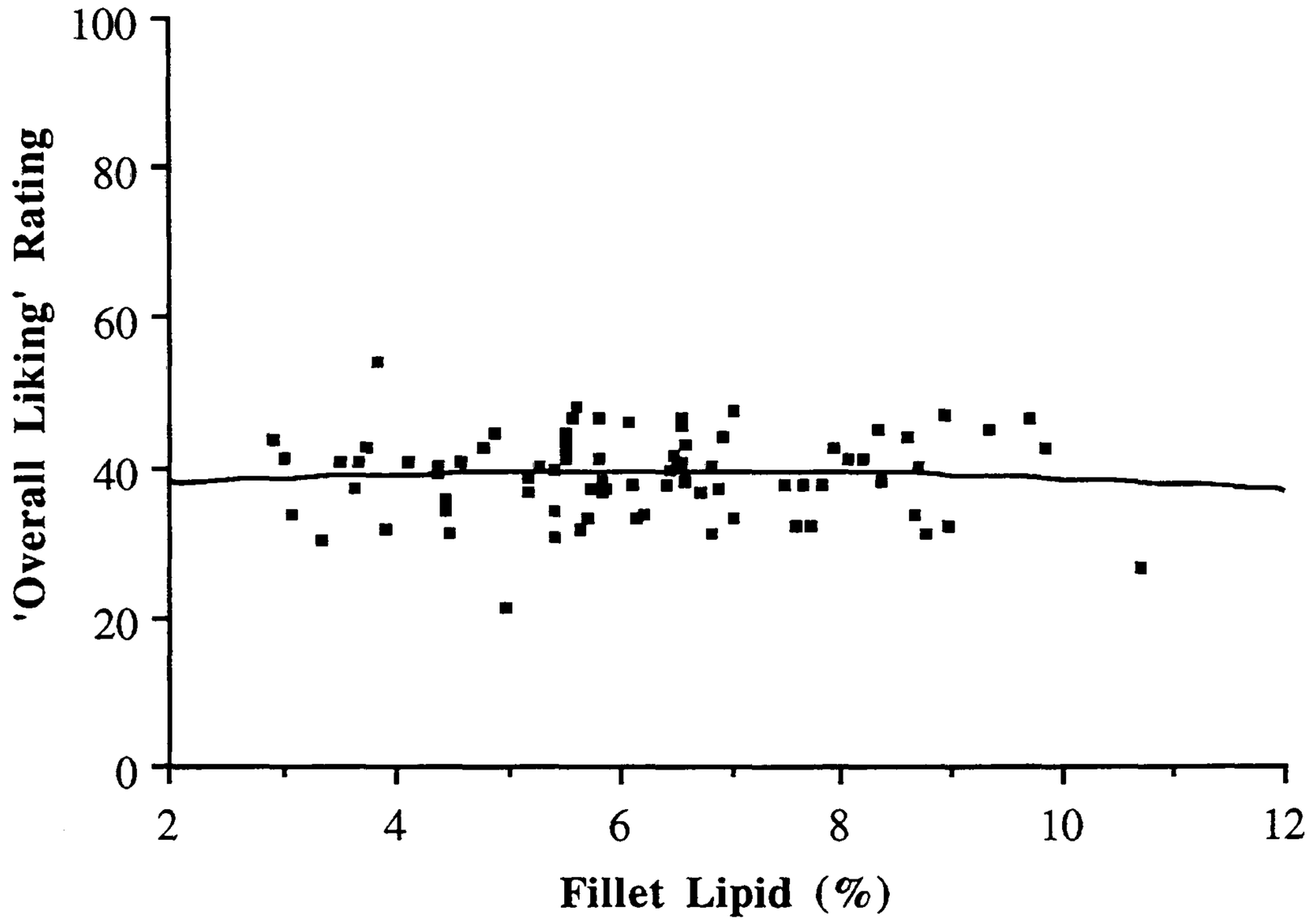

Figure 3.3.36: Relation between *overall liking* and lipid content.
$y = -0.08x^2 + 1.08x + 35.90$, $r^2 = 0.003$

Finally the *overall flavour* ratings were plotted against the *overall liking* ratings and a regression line fitted to the data (figure 3.3.37). The correlation coefficient of r=0.931 indicated that there was a significant correlation between the two ratings ($p<0.001$). This was the strongest correlation found in the trial on the fresh cooked fish.

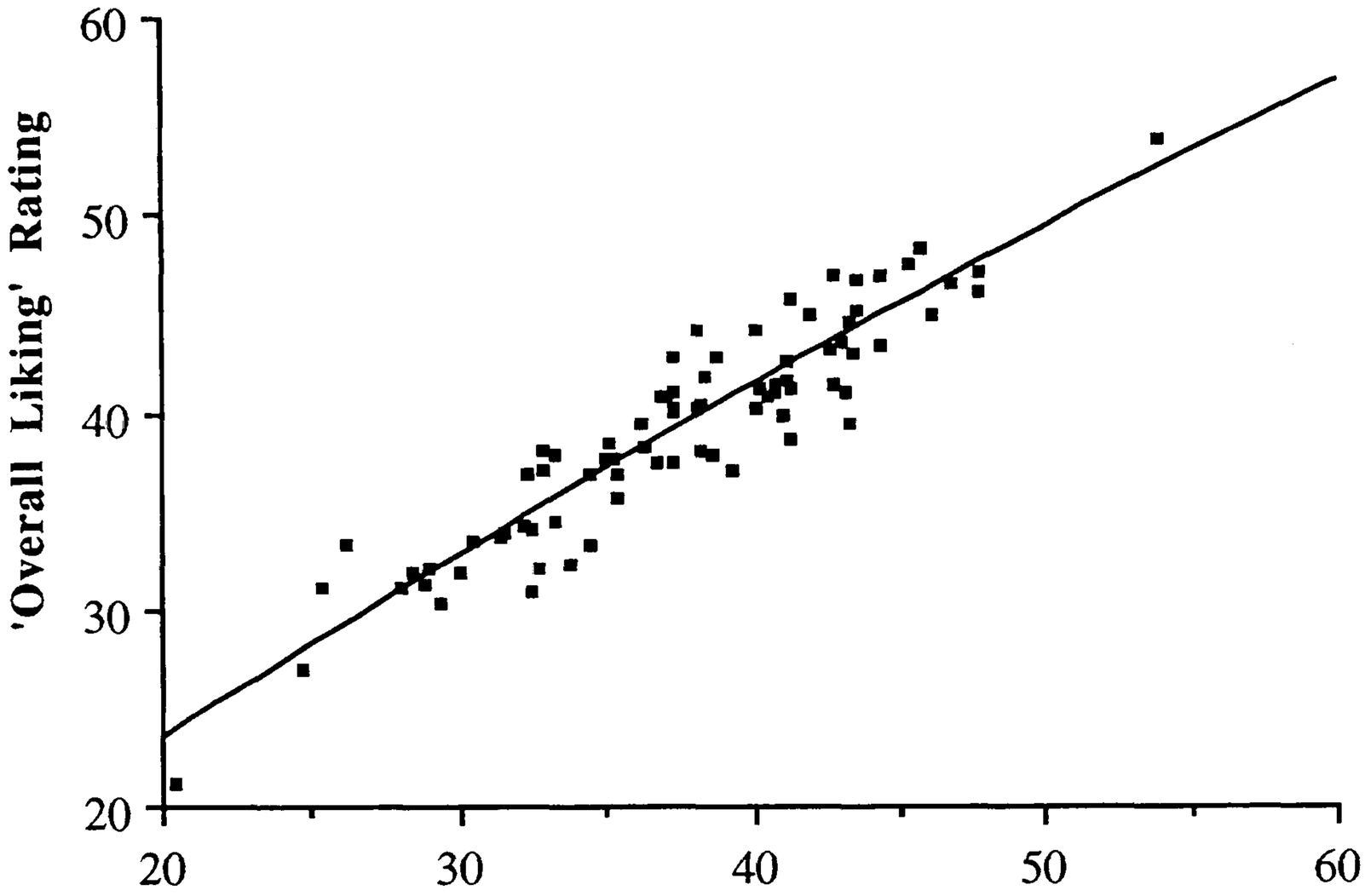

Figure 3.3.37: Relation between *overall liking* and *overall flavour* .

$y = -0.003x^2 + 1.08x + 3.00$, $r^2 = 0.867$

3.3.4.4 Turning Points of Fresh Salmon Attributes

From the equations of the regression lines, it was possible to calculate where the maximum or minimum ratings for each attribute lay. This was done by differentiation to identify where the slope of the curve was zero. Table 3.3.26 shows the results of this analysis. Although very few of the attributes were significantly affected by lipid content, there appears to be a turning point at about 7% lipid for several of those which were affected.

Table 3.3.26 Turning points of the texture and flavour ratings significantly affected by lipid levels for the fresh cooked fish.

Attribute	Lipid Content (%)	Max. or Min.	Sig. of Curve
Firmness on Cut	-	-	ns
Disintegration on Cut	-	-	ns
Slimy on Bite	-	-	ns
Firmness on Bite	3.7	Max.	<0.05
Moistness on Chew	7.6	Max.	<0.05
Sticky on Chew	-	-	ns
Gelatinous on Chew	-	-	ns
Chewiness on Chew	5.8	Max.	<0.05
Fibrous on Chew	7.6	Max.	<0.05
Cohesion on Chew	6.2	Max.	<0.05
Dissolubility on Chew	-	-	ns
Fishy Flavour	-	-	ns
Bitter Flavour	2.39	Min.	<0.05
Creamy Flavour	-	-	ns
Seaweed Flavour	8.6	Max.	<0.1
Oily Flavour	30.5	Max.	<0.01
Sour Flavour	2.9	Min.	<0.05
Sweet Flavour	-	-	ns
Metallic Flavour	-	-	ns
Earthy Flavour	-	-	ns
Stale Flavour	-	-	ns
Salty Flavour	-	-	ns
Overall Flavour	-	-	ns
Overall Liking	-	-	ns

3.4 Discussion

Wiseman (1993) showed the effect of pre-slaughter fasting and exercise on the texture and flavour of rainbow trout. Exercise had some effects on texture, but none on flavour. Fasting over an 18 day period also affected texture (decreased *stickiness* and increased *gelatinous* character, *sliminess* and *flakiness* with starving) and had an effect on four flavour attributes (less *fishy*, less *sour*, more *stale* and less *earthy* with starving). The fasting also produced a slight drop in total lipid content of the white muscle (1.38% compared to 1.69% in the fed fish).

A fasting period of up to two months resulted in a greater loss of total lipid in rainbow trout (Johansson and Kiessling, 1991). With total lipid values of 5.3%, 4.2% and 3.4% for white and red muscle (no starve, 1 month starve and 2 months starve respectively) effects on odour, flavour and texture were found. Total odour decreased with starvation, as did *acidulous* odour. *Bitter* flavours also decreased and starved fish were less *juicy*. The low number of significant effects found in this work was almost certainly due to the very low number of samples in each group (n=8 compared with n=20 with Wiseman, 1993).

Stress has been shown to affect muscle properties including the strength of the muscle fibres (Jerrett *et al.*, 1996). Slaughter of the fish is a stressful period for them, with high levels of activity during the crowding and killing. Post-mortem handling and processing may also affect texture. After slaughter, fish are placed in bins of ice slurry to chill them and the rate of this cooling affects the rate at which the enzymes in the fish slow down their reaction rates. Enzyme activity within the fish post-slaughter may result in the degradation of the flesh, thus affecting the texture (Huss, 1976 cited in Johansson and Kiessling, 1991). After the initial chilling, it is important that the fish are kept at the same temperature in order to minimise enzyme activity. However,

this procedure cannot be maintained during smoking, when the fish have to be exposed to a temperature of 24°C or higher.

The texture of fish may also be affected by handling whilst the fish are in rigor (pers. comm. A. Dingwall, Pinneys of Scotland, Dumfriesshire, United Kingdom, and pers. obs., 1996). If the fish are bent from one shape into another when their muscles are in rigor, the muscle fibres tear. The tearing affects the texture of the flesh, making it feel softer and causing the appearance of gaps on filleting.

The storage conditions of the fish after processing affects both the texture and flavour. Frozen fish were shown to be significantly less *juicy*, less *rancid*, to have a less *muddy* taste and odour and to have less *fresh* odour than fresh fish (Johansson and Kiessling, 1991). However, no differences in eating quality were found by the authors between fish stored frozen at -18°C for three months and six months. Fish stored on ice at 0°C showed a much more marked effect of storage, with declining *fresh* taste, *acidulous* taste, *fresh* odour and *juiciness* after two weeks of storage.

The above findings show the important effects of ante- and post-mortem treatments on eating quality. Stress at slaughter affects instrumentally-measured texture, as does post-slaughter handling. Storage conditions also will result in changes in eating quality. Differences in husbandry between groups could affect the texture and flavour of the fish. It was therefore essential to treat all of the fish in this experiment in the same way in order to minimise these effects..

The natural variation in a population of animals will always produce a distribution of a specific trait. In this experiment the trait investigated was the level of lipid in the white muscle of the fish. The degree of variation observed was much larger than that found in farmed mammals and poultry. This was partly explained by the variation in size of the fish at slaughter and partly by the comparatively short period for which

salmon have been farmed. The short period of domestication has not allowed farmers to select for specific traits in the farmed fish to minimise variation. In comparison, cattle and pigs have been farmed for centuries and selection has been practised for a long time. However, the large degree of variation in white muscle lipid in one population of fish has allowed this experiment to be tightly controlled for other factors which might have affected the eating quality of these fish.

By growing all the fish in the same cage many husbandry and handling factors were controlled. The pre-slaughter handling procedure was the same for all fish. No food was given to the fish for seven days prior to slaughter and the whole cage was exposed to the same tidal currents. Thus it can be said that all the fish were exposed to the same fasting period and level of exercise prior to slaughter.

The fish were crowded in the pen in order to facilitate catching. The last fish to be caught would have been more stressed than the first ones, due to the duration of the crowd. However, there was no evidence to show that the order of the killing of the fish was related to their lipid content. Therefore it can be assumed that the crowding stress would have been randomised between all groups and that the effect of stress on texture and flavour was controlled for in this trial.

After slaughter all the fish were placed into the same bin of ice slurry and were eviscerated at the same time. This gave a similar cooling rate for all the fish, but, again, any effects due to cooling rate were randomised between the groups. All the fish were eviscerated before they went into rigor and were repacked randomly in ice. After filleting, the fresh fillets were all frozen to the same temperature and stored together in one freezer and the fillets to be smoked were kept together as a batch under the same conditions.

The smoked fish were not cooked before presentation to the taste panel. However, the fresh fish were and this was another carefully controlled stage in the experiment. Johansson *et al* (1992) showed the importance of cooking conditions on the eating quality of rainbow trout. The method of heating the fish was important, but the final internal temperature of the fish had a greater effect on the eating quality. Fillets of different thicknesses obviously heat at different rates and so great care had to be taken to measure the internal temperature of each individual fillet and to remove each one at the set temperature.

By the nature of this experiment, the controls were very tight. Thus it appears to be safe to draw conclusions on the effect of the levels of lipid in the fish on the eating quality of both the fresh and smoked fillets.

The use of texture and flavour profiles derived from a trained taste panel to describe groups and individuals resulted in detailed analyses of the eating quality of the fish. It allowed the groups to be differentiated between and trends between individuals to be observed. By using the trained taste panel, where individuals' responses to different stimuli are known and can be quantified, the results are meaningful even when using a small number of samples.

Without previous training, the taste panel would have been less sensitive and therefore a much larger number of samples would have had to be used in order to identify real differences. This would have been an expensive and time consuming operation, if done properly. If the number of samples used was small, it would be unlikely that significant differences would have been found between the groups, even if there were effects.

The members of the taste panel in this experiment were normally used for the assessment of the eating quality of red meat and poultry. With the meat from these

species of animals, texture is a very important factor. This could explain why so many texture attributes were identified by the panel compared to other panels—for example in the work of Johansson and Kiessling (1991), where only three attributes were found for texture.

Splitting up the texture attributes into three groups— on cutting, on first bite and on chewing— initially seems very pedantic, but the whole sensory experience had to be investigated. At first sight, the texture of the fish might not appear to be of great importance. Rasekh *et al.* (1970) highlighted the importance of visual appearance and the flavour as the two factors giving 80% of the influence of the consumer's decision to buy a fish product. However, salmon tends to be a soft fleshed fish and this renders it susceptible to damage during processing. It is therefore important to gain as much information as possible on all aspects of texture.

3.4.1 Texture of Smoked Salmon

Texture ratings in smoked salmon followed an expected pattern. *Firmness* of the samples decreased with increasing lipid content on cutting, first bite and chewing. It appeared that the ratings from cutting and the first bite were closely linked to each other, both reaching a minimum rating at approximately 9% lipid. However, *firmness* on chewing appeared to be unrelated to these two.

The *cleanliness* rating of the cut increased with increasing lipid over the range 2% to 6%, before decreasing at the lipid levels above 8%. The description of this attribute showed that the rating increased as fewer fibres were cut through. As the lipid level increased from 2% to 6% the binding between the fibres may have become softer so that the fibres tended to part more easily leaving a rougher cut surface with less fibres cut cleanly through. Above 8%, however, the fibres themselves may have become softer and easier to be cut through. This would have led to an appearance of a

cleaner cut at the higher lipid levels. Further investigation is required to see if this is the case. Such a study would be easy to carry out using a microscope to study the effect of the cutting on the fibres.

Oily and *slimy* sensations on the first bite increased with increasing lipid content. Both of these sensations are associated with lipids and this may imply that when the muscle fibres are broken during biting, more free oil is released from the higher lipid fish. This may have processing implications, as trimming and slicing of smoked salmon are routine operations which break the muscle fibres. Free lipid in the packs around smoked salmon is unsightly to the consumer. If fish with a very high lipid content are more susceptible to the release of free lipid on trimming and slicing this will lead to a less desirable product after packing.

Texture on chewing was strongly affected by lipid content. The increase of the *jellified* sensation was expected, as this texture is strongly associated with lipid level. The increase in *dissolubility* confirms the results of the *clean-cut* ratings and the implication that as flesh lipid increases, the muscle fibres fall apart more easily.

The increase of the sensation of a *moist* texture with increasing lipid was interesting. As was shown in the previous chapter, moisture and lipid level are inversely related — thus the higher lipid fish would have contained less moisture. It can therefore be concluded that lipid content plays a more important role in the perceived moisture of the flesh than the actual level of moisture. This may be related to the release of lipids from the fibres on chewing. In this sense the attribute *moist* used in fish is very similar to the attributes *succulence* or *juiciness* used in red meat. These attributes are also dependent on the lipid content.

There was no effect of lipid content on the *cohesion* of the sample after chewing, prior to swallowing. However, from the spread of the data for individual fish it was

obvious that there was variation in this attribute. Further work needs to be carried out to determine what affects this attribute.

3.4.2 Texture of Cooked Salmon

The effect of lipid content on the texture of the cooked fish was not so great as that seen in the smoked fish. There was no effect of lipid group on either of the texture attributes at cutting. *Firmness* on the first bite was not affected by lipid group, a result also found by Kestin *et al.* (1995b) on fresh cooked rainbow trout. However, plotting individual ratings against the lipid content showed a weak correlation. As expected, the perceived *firmness* decreased with increasing lipid.

On chewing, *moistness* was affected by lipid groups. As with the smoked fish there was an increase in *moistness* ratings with increasing lipid. This result was similar to that observed by Kestin *et al* (1995b). Again this was probably due to the increase in lipids released on biting resulting in a more *moist* sensation. The *moistness* attribute discussed here may be similar to the *juiciness* discovered by Johansson and Kiessling (1991). These authors found a decrease in *juiciness* with starvation, which had resulted in a reduction in total lipid. This was supported by the findings of the current experiment.

Chewiness was also found to increase with increasing lipid content to about 6% lipid. Then the ratings decreased again. It would be expected, from the decrease in *firmness* with increasing lipid, that the samples would become less *chewy* as well. The increase at first may be due to an interaction with lipid and another, unknown component of the muscle, with lipid becoming more important above the 6% level. Kestin *et al* (1995b) found no effect on *chewiness* in rainbow trout.

Fibrous texture increased at low lipid levels and then reached a maximum. The difference between the lipid groups was very small, though significant between the 2-5% group and the rest. Thus, although the maximum rating from the graph was calculated to occur at 7.6% lipid, there was effectively no difference between the ratings above 5% lipid. This may have occurred if the fibres in the fish with higher lipid levels tended to split apart on chewing rather than to break up. This would give a more fibrous sensation. However, owing to the nature of the muscle fibres and the soft texture of the flesh, the ratings for *fibrous* texture were not high.

Cohesion of the sample after chewing was affected in the fresh cooked fish. Individual ratings showed that the *cohesion* increased to a maximum at 6.2% lipid and then decreased again. This implies that another factor affects *cohesion*, possibly the same factor which affected the *cohesion* on chewing of the smoked samples, which were also unaffected by lipid content.

The reason for the small effects of lipid content on the texture of the fresh cooked fish compared to the smoked fish was not clear. As the fillets used for the two trials were from the same fish, it can be concluded that the effect was real and not an artefact of the experiment.

Kestin *et al* (1995b) found that only one texture attribute out of the nine described by the taste panellists was affected by lipid content in cooked rainbow trout. This was supported by the current conclusion that lipid content does not play a major role in affecting the texture of the cooked fish. The texture of cooked fish seems to be highly correlated to muscle pH (Love *et al.*, 1974), with decreased texture after cooking associated with a higher muscle pH measured one day after slaughter. However, the smoked product texture was clearly affected by the level of lipid in the flesh. This highlighted the difference in the nature of the smoked and cooked products.

Analysis of the regression curves fitted to the data showed that many of the texture attributes detected reached maximum or minimum ratings in the range investigated (2% to 10% lipid). In the smoked fish both the minima and maxima were concentrated in the 8% to 10% lipid range. This implies that this area of lipid content is of great importance to the overall texture. *Jellified* and *dissolubility* texture descriptors on chewing reached maxima at 18%, outside the range investigated and *firmness* on chewing decreased to a minimum at 93% — almost pure lipid!

However, in the fresh cooked fillets the turning points were much lower, ranging between 4% and 8% for the texture attributes. This again clearly highlights the differences between the two products. Each of the attributes that was affected by lipid content reached maximum values in the range investigated. As the attributes were all associated with increasing overall texture, apart from *moistness*, this implied that the maximum overall texture of the fillets was achieved in this range.

3.4.3 Flavour of Smoked Salmon

The flavour of the fish is perhaps of most importance to the consumer (Rasekh *et al.* 1970). It is the final part of the eating experience and leaves the most lasting memory. Six flavour attributes were identified in the smoked fish by the taste panellists. *Fishy* flavour was rated highly throughout the lipid range, although the ratings were lower in the lower lipid group. This was confirmed by the scatter plot, which showed that the maximum fishy flavour was achieved at about 8.6% lipid, with the flavour starting to decrease above 10%. This implies that *fishy* flavour is carried by the lipid, but at high levels of lipid it starts to be masked.

Salty flavour ratings were very high in all samples. Analysis of the lipid group means showed that the flavour decreased rapidly with increasing lipid, which was supported by the scatter plot. Salt is used during the curing process and this is probably the

cause of the strong flavour. The lower lipid fillets would have had a higher moisture level and this may have caused more salt to enter the fillet during curing. This would have resulted in the higher ratings observed for the low lipid fillets. Further work is needed to measure the levels of salt in the fillets and to try to correlate them with both moisture and lipid levels and with the *salty* flavour attribute.

Smoky flavour is obviously of great importance to the product. However, it was unaffected by the level of lipid in the fillets. This was unexpected as it was thought that the flavours in the smoke would bind to the oils in the fish and result in the fish with higher lipid contents having more flavour. Apparently this is not the case and the *smoked* flavour must be carried in a different way.

Sour flavour was affected by the lipid group, but the effect did not appear progressive. However, using the scatter plot a correlation between the flavour and lipid content appeared, with the sour flavour decreasing with increasing lipid. The ratings for this flavour were very low, which meant that the flavour was hard to detect. This would have made it difficult for the panellists to distinguish the grades of flavour precisely.

Oily flavour, as expected, showed an increase with increasing lipid. The minimum flavour was observed at approximately 4% lipid, but above this point the flavour increased rapidly. This may have resulted from the lipid being released from the higher lipid fish more easily on chewing. However, at the higher lipid levels there was a lot of variation in the ratings between individual fish. This may have been caused by an increase in another flavour,— *fishy* flavour was the strongest flavour at this point— resulting in the masking of the *oily* flavour in some fish.

Metallic flavour ratings were very low as the flavour was hard to detect, or was not present. No effects of lipid content were found on this flavour. It is probably not generally an important flavour in smoked salmon.

3.4.4 Flavour in Cooked Salmon

In the cooked fish, many more flavours were identified by the taste panel than in the smoked fish. However, most of these ratings were very low and were generally unaffected by the level of lipid in the fillet. Only *fishy* flavour ratings were high, but this was not affected by the level of lipid in the flesh, unlike smoked fish and rainbow trout where *fishiness* increased with increasing lipid content (Kestin *et al.* 1995b).

Seaweed and *sour* flavours both showed an increase with increasing lipid, despite low ratings. The effect on *sour* flavour was the opposite of that observed in the smoked fish, where the flavour decreased with increasing lipid. In rainbow trout there were no effects of lipid content on the *sour* flavour (Kestin *et al.* 1995b). However, as with the smoked fish and rainbow trout, *oily* flavour showed an increase with increasing individual lipid content.

Earthy flavour, a spoiling flavour in trout, was detected at very low levels and found to be affected by lipid groups. This was also shown by Kestin *et al.* (1995b), but not by Johansson and Kiessling (1991). However, the attribute did not appear to be affected by the individual lipid level as there was a high degree of variation which prevented a clear correlation being found.

3.4.5 Overall Ratings

The *overall flavour* ratings for smoked salmon increased with increasing lipid. A very strong correlation was found between the two, which appeared almost linear although a stronger correlation was found for a quadratic regression line. From the other flavour attributes, it would appear that the most important contributors to *overall flavour* were *fishy* and *oily* flavours, with a low *salty* flavour.

However, the *overall flavour* of the cooked fish was completely unaffected by the level of lipid. From the variation in the data, it would appear that another, unknown, factor plays an important role in the *overall flavour* of the cooked fish.

As with texture, the analysis of the turning points of the regression curves fitted to the data showed that several of the flavour attributes reached either maxima or minima in the range of lipid contents investigated. However, in smoked fish, *overall flavour* increased well outside the range investigated. This strongly implies that the flavours in smoked salmon are associated with the lipid fraction, either being lipids themselves or compounds bound to them.

The *overall liking* ratings allowed the taste panellists to express their own opinion of the food and helped to gauge which factors affected the overall eating experience. In smoked fish there was a very strong correlation between increasing lipid and increasing liking, with the maximal liking rating calculated to occur with a fish of 56% lipid! However, this is most likely to indicate that the relationship between *overall liking* and lipid content is linear rather than quadratic. From the attributes previously discussed, it could be seen that the panellists liked a less *firm* flesh, with a *moister*, *oilier* texture. Flavours which were liked were *fishy* and *oily*, but strong *salty* flavours were disliked. The *overall flavour* of the fish was also very important for the *overall liking*, showing a very strong correlation ($p<0.001$).

In contrast, the *overall liking* ratings of the fresh cooked fish were unrelated to lipid content. This differed from the findings of Kestin *et al.* (1995b) showed a strong effect of lipid content on the *overall liking* of cooked rainbow trout ($p<0.001$). However, in salmon a very strong correlation was found between *overall liking* and the *overall flavour* ratings ($p<0.001$). This indicated that flavour was a very important factor affecting the *overall liking* in the fresh cooked fish. This supports the findings of Rasekh *et al.* (1970) who found that flavour was more important than

texture in cooked fish. However, further work is required to identify the factors which affect the flavours in cooked salmon as they are clearly different from those in trout.

From this experiment it is very clear that the level of fillet lipid has an effect on the eating quality of both fresh and smoked salmon. However, the *overall flavour* and *overall liking* are only affected in smoked salmon, with both attributes increasing greatly as lipid content increases. This has significant implications for the industry, because at present the trend is to try to encourage farmers to produce fish with lower lipid contents as higher lipid fish are more susceptible to downgrading during current processing practises due to their softer texture. However, this appears to run the risk of decreasing the flavour and the liking of the product. Thus, the technological problems of handling the softer fish will have to be overcome if the higher levels of flavour which have been demonstrated to be associated with the high lipid fish are to be maintained.

Chapter 4

The Effect of Activity at Slaughter on White Muscle pH and Colour

4.1 Introduction

In the previous chapter it was shown how the texture and flavour of the fish may be affected by the levels of fillet lipid. However, although these are important variables of overall quality, the appearance of the product is also of extreme importance (Rasekh *et al.* 1970).

In salmonids the colour of the flesh is used as a cue by consumers in making their decision to buy the product. Therefore the specifications of companies buying fresh salmon from farms include sections on the colour of the flesh. These insist on the flesh attaining certain levels of colour and also a degree of uniformity of colour across the fillet.

In red meat species, it has been shown that stress or activity at slaughter affects the colour and texture of the flesh post-slaughter (Warriss, 1996). The increased activity results in rapid acidification of the flesh causing changes in the characteristics of the muscle proteins, which lead to the changes in colour and texture.

High levels of 'stress' or activity at the slaughter of fish have been shown to result in an increase in the rate of acidification of the flesh post-slaughter (Erikson, 1997; Jerrett *et al.*, 1996). It could therefore be expected that changes in the muscle protein structure may occur in a similar way to that observed in red meat species, resulting in the masking of the flesh colour. High activity also reduces the time the fish take to enter rigor (Lowe *et al.*, 1993), another quality parameter.

The work presented in this chapter has as its aim the investigation of the effect of different levels of activity at slaughter on the flesh colour of salmonids. Several levels of activity may result pre-slaughter during the application of current commercial slaughter methods and model systems. Fish anaesthetised with

commercial anaesthetics may be classed as 'low activity' fish. 'Highly active' fish result from a model system, using electricity to stimulate the muscles into high levels of activity. This gives a reproducible method of 'exercising' the fish. A range of other commercial methods of slaughter from percussion stunning to carbon dioxide anaesthesia result in intermediate levels of activity at slaughter.

For this study, a series of three experiments was carried out. The first two experiments used rainbow trout as model animals to determine if there were effects and where they may occur after slaughter. The final experiment was carried out on Atlantic salmon, as this species is of the greatest economic value.

Apart from colour, the experiments also investigated the drop in muscle pH post-slaughter and the effects of activity on the time the fish took to enter *rigor mortis* and for this to resolve. The time to rigor is of great interest to the industry as when the fish are in rigor they cannot be handled without damaging the flesh.

4.2 Method

4.2.1 Experiment 1

Large all-female rainbow trout were raised in the same 7m diameter tank for 6 months at Whitebrook Fish Farm, Gwent, U.K., being fed the trout diet Vextra Gold (EWOS Ltd., Bathgate, West Lothian, U.K.) containing 50ppm astaxanthin. All fish had been raised together at Whitebrook since transfer from Houghton Springs Fish Farm, Dorset at 100g. Thirty fish of mean weight 1.5kg were netted out of that tank and randomly allocated to two tanks 3m in diameter and 1m in depth of water, so that there were ten fish in one tank (tank A) and twenty in the second (tank B). The fish were then left for three days to recover from the handling and transfer stress. During this time the fish received no feed — a standard commercial pre-slaughter procedure.

4.2.1.1 Slaughter

After the recovery period the fish were slaughtered as following:

i) Anaesthetised

The water level in tank A was reduced by half and the anaesthetic 2-phenoxyethanol slowly added, with care taken not to disturb the fish. The anaesthetic was added to the inflow water so it was distributed throughout the tank. When the concentration of anaesthetic was 0.2ml/l, the inflow water was turned off and the fish left to succumb to the anaesthetic.

Approximately ten minutes after the application of the anaesthetic all movement had stopped in the tank. The fish were then netted out of the tank individually and killed

by a blow to the head with a priest. After death the fish were filleted and the fillets tagged ready for measuring.

ii) Netted Then Slaughtered

The water level in tank B was reduced to one quarter of the original level. Ten fish were netted out individually as rapidly as possible and killed by a blow to the head with a priest, filleted and tagged ready for measuring.

iii) Electro-stimulated

The final ten fish in tank B were killed by a blow to the head, had their hearts excised and were then electro-stimulated. One electrode was connected to the tip of the caudal vertebrae using a crocodile clip. The second electrode was attached to a fifteen centimetre long steel needle which was inserted dorso-ventrally through the musculature of the fish just behind the head as close to the backbone as possible (figure 4.2.1). The electrodes were connected to a low voltage electro-stimulator control box (J.S. Engineering, Norwich, U.K.). The fish then received two one minute bursts of low voltage pulsed d.c. (94V at 14.3Hz). The stimulation resulted in large amounts of muscle activity, forming a model of a highly active fish. After stimulation the fish were disconnected from the electrodes, filleted and tagged ready for measuring.

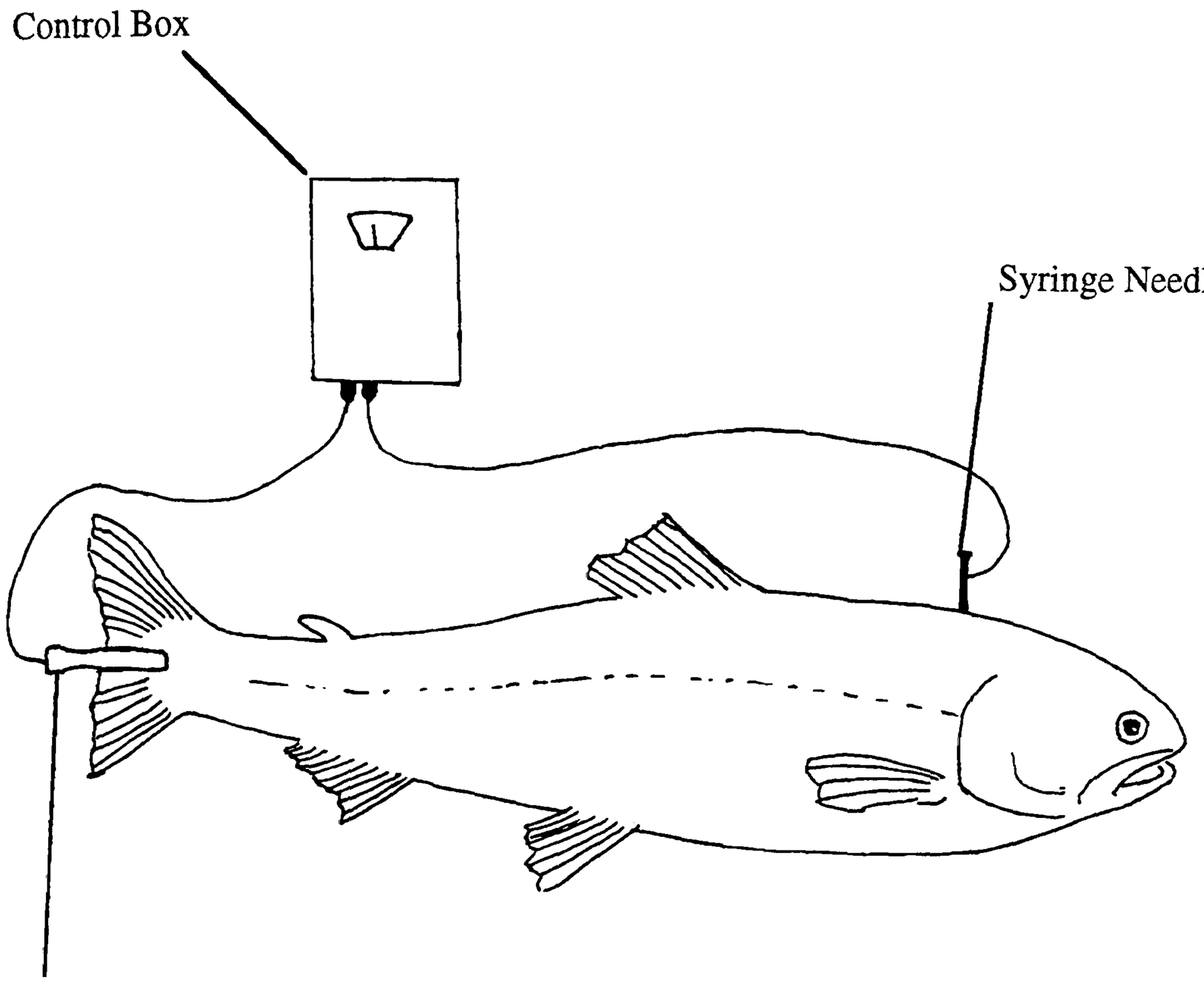

Figure 4.2.1: Set up for the electro-stimulation of the trout.

4.2.1.2 Measurements

The fish were measured for temperature, pH, and colour in the region of flesh on the fillet between the dorsal fin and the midline (figure 4.2.2). This area was chosen as it was in the middle of the fillet, so any colour problems would be particularly noticeable to the consumer. It is also a large area of flesh, which can be easily defined for repeated measurements. Prior to each series of measurements all the fillets were wiped with absorbent paper to remove blood and muscle exudate which could have interfered with the measurements.

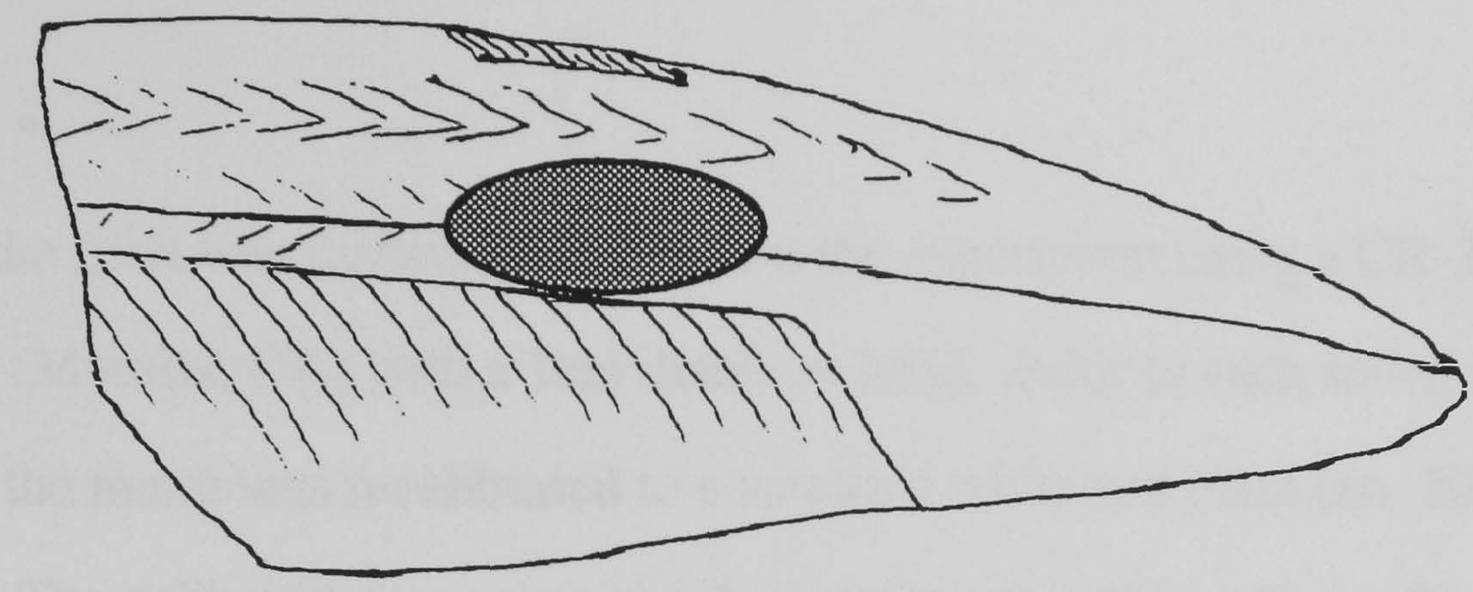

Figure 4.2.2 Region of the fillet measured for temperature, pH and colour.

i) Temperature

The temperature of each fish was measured using a thermocouple probe attached to a digital thermometer (Whatman, UK). The tip of the probe was carefully inserted into the fillet so that it penetrated to about half the depth of the flesh.

ii) pH

The pH of the fillet was measured *in situ* using a PHM8 Portable pH Meter (Radiometer, Copenhagen, Denmark) with a spear combination pH electrode (LoT406-M6-DXK-S7/25, Mettler Toledo). Before each series of measurements the meter was calibrated using standard buffers at pH 5.00 and 7.00 (190354F and 192403F BDH, U.K. respectively), which were kept at room temperature until the fish were placed in the chiller, when they too were kept at 4°C. This enabled the meter to be calibrated, allowing for the sample temperature. The tip of the glass electrode attached to the meter was carefully inserted into the fillet until it had penetrated to about half the thickness. The electrode was held in place until the

readings had stabilised and the pH was then read. For each measurement a new hole was made in the fillet, as it was found that misleading results were given if an old hole was reused. This was probably caused by the collection of drip in the holes.

iii) Colour

The colour of the fillet was measured throughout the experiment using a CR-200 Chroma Meter (Minolta, UK) with a 1cm diameter head. Prior to each series of measurements the meter was recalibrated to a standard white test plate (no. 20232754, Minolta, UK). The calibration was then checked against the white plate and a standard red plate (no. 101663, Minolta, UK). The colour of the fillet was measured according to the CIELab (1976) methods. This resulted in the machine giving three values for each sample — L*, a* and b*. At each measurement point three measurements were taken of each fillet, with the head of the meter being moved slightly between each. This was to allow for any positional errors. The muscle contained a lot of myosepta which were white and would tend to lighten the measured colour. By moving the head between readings this effect could be minimised.

From the L*, a* and b* values the colour of the flesh could be described. The L* value gave information on the lightness of the flesh. The a* and b* values were used together to determine the colour and opacity of the flesh.

The colour of the flesh was defined using the angle of hue. This was determined using the formula:

$$\text{Angle of Hue} = \text{Arctan}\ (\ b^* / a^*\ )$$

As the angle approached 0° the colour became more red. As the angle went towards 90° the colour was more yellow .

The opacity of the flesh was defined using chroma. The chroma was determined using the formula:

$$\text{Chroma} = \sqrt{\left((a^*)^2 + (b^*)^2 \right)}$$

The greater the measured chroma the more opaque the sample.

The angle of hue and chroma were calculated for each of the three measurements taken from the fillet at each point in time. The mean L*, angle of hue and chroma were then determined for each individual fillet at each time.

At the end of the storage period the fillets were placed flat on an evenly lit white table. The fillets were wiped with absorbent paper and five assessors asked to score the fillets for colour using a Roche colour card (F. Hoffmann-La Roche, Basel). The fillet scale of the card was used, with scores ranging from 11 to 18. The mean score for each fillet was calculated.

iv) Gaping

Finally the fillets were run, skin side down, over a right angle bend using the same force for each fillet. This caused the appearance of gaping in all fillets, being a simulation of very rough handling. The degree of gaping was scored by an assessor using a four point subjective scale ranging from 0 (nil) to 3 (maximum). A score of three resulted from almost all of the myosepta along the fillet having broken and some gaping to have occurred within the myotoma.

4.2.1.3 Storage

After the initial measurements had been taken, the fillets were placed one layer deep in polystyrene fish boxes on a sheet of plastic over a thick layer of ice. A second sheet of plastic was placed over the fillets and a layer of ice placed on top. Ten fillets were placed in each box. The boxes were then stored in a chiller which was kept at 4˚C. They were stored there for four days post-slaughter and were measured at intervals: approximately 1, 2, 6, 8, 22, 33, 45, 57, 69 and 75 hours after slaughter, although times differed slightly for each group due to different slaughter times.

4.2.2 Experiment 2

Following the results of experiment 1, a follow-up was planned to make a trial run with the anaesthetic AQUI-S™. This experiment used the same initial population of rainbow trout as the first experiment, although the mean weight had increased to approximately 2kg. This experiment was conducted seven months after the first experiment using the same facilities at Whitebrook Fish Farm. During this time the trout had matured and been stripped ten weeks before the experiment started. The fish had recovered their flesh colour when the experiment started, but there may have been some residual effects of maturation on other flesh properties.

Forty fish were netted into a single tank 3m diamter tank with a 1m depth of water and kept for three days to recover from the handling and transfer stress. On the first day after transfer they were fed two meals to help replenish energy reserves. They were then starved for the next two days leading up to slaughter.

4.2.2.1 Slaughter

On the day of slaughter the level of water in the tank was halved to gently crowd the fish, without disturbing them. The volume of the water was estimated and the volume of the anaesthetic AQUI-S™ (Fish Transport Systems Ltd., New Zealand) required to make a concentration of 17ml per thousand litres (17µl / l) in the tank calculated. This volume was mixed with ten times that volume of water to form an emulsion. The emulsion was added to the tank using a domestic garden sprayer as directed by instructions from Fish Transport Systems Ltd., spraying the emulsion into the inflow water so that it was mixed throughout the tank.

The fish were then left undisturbed to be anaesthetised. When all movement in the tank had ceased the fish were individually netted out of the tank and hit on the head using a priest. The fish were killed in two batches of twenty. From the first twenty, ten fish were selected at random, had their hearts removed and were electro-stimulated as in the first experiment. The remaining ten from the first batch were left unexercised. Both groups of fish were filleted, tagged and measured as in the first experiment. They were then stored on ice for three days under the same conditions as in experiment 1. Further measurements of temperature, pH and colour were taken at regular intervals. Colour was measured at each point using the chroma meter and using the Roche colour card 24 hours and 72 hours post-mortem. The temperature, pH and chroma meter readings were carried out at 2, 4, 6, 8, 10, 12, 20.5, 24, 30, 34, 48, 56, 72 and 96 hours after slaughter.

The second batch of twenty anaesthetised fish were removed from the tank and killed by a blow to the head with a priest. Again ten fish were selected at random and were electro-stimulated after their hearts had been removed. The other ten fish were left unexercised. This batch of twenty fish were left uneviscerated and were used for rigor measurements.

4.2.2.2 Rigor Measurements

The degree of rigor of the fish was determined using a very simple apparatus. The fish was clamped horizontally so that the head and body up to the posterior margin of the dorsal fin were supported (figure 4.2.3). The rest of the body and the tail were allowed to droop unsupported over a right angle bend. The angle formed between the right angle bend and the tip of the tail was determined to an accuracy of 5°. The fish was then turned over so that the tail drooped the other way and the measurement repeated. The mean angle of droop was calculated for each fish to give information on the state of rigor of the fish. This was done to allow for any predisposition towards bending one way rather than the other way that the fish may have had, which was especially important once rigor had set in, as the fish were often slightly bent during storage.

Rigor measurements were carried out at 0, 4, 8, 12, 20, 24, 34, 48, 56, 72 and 96 hours after slaughter.

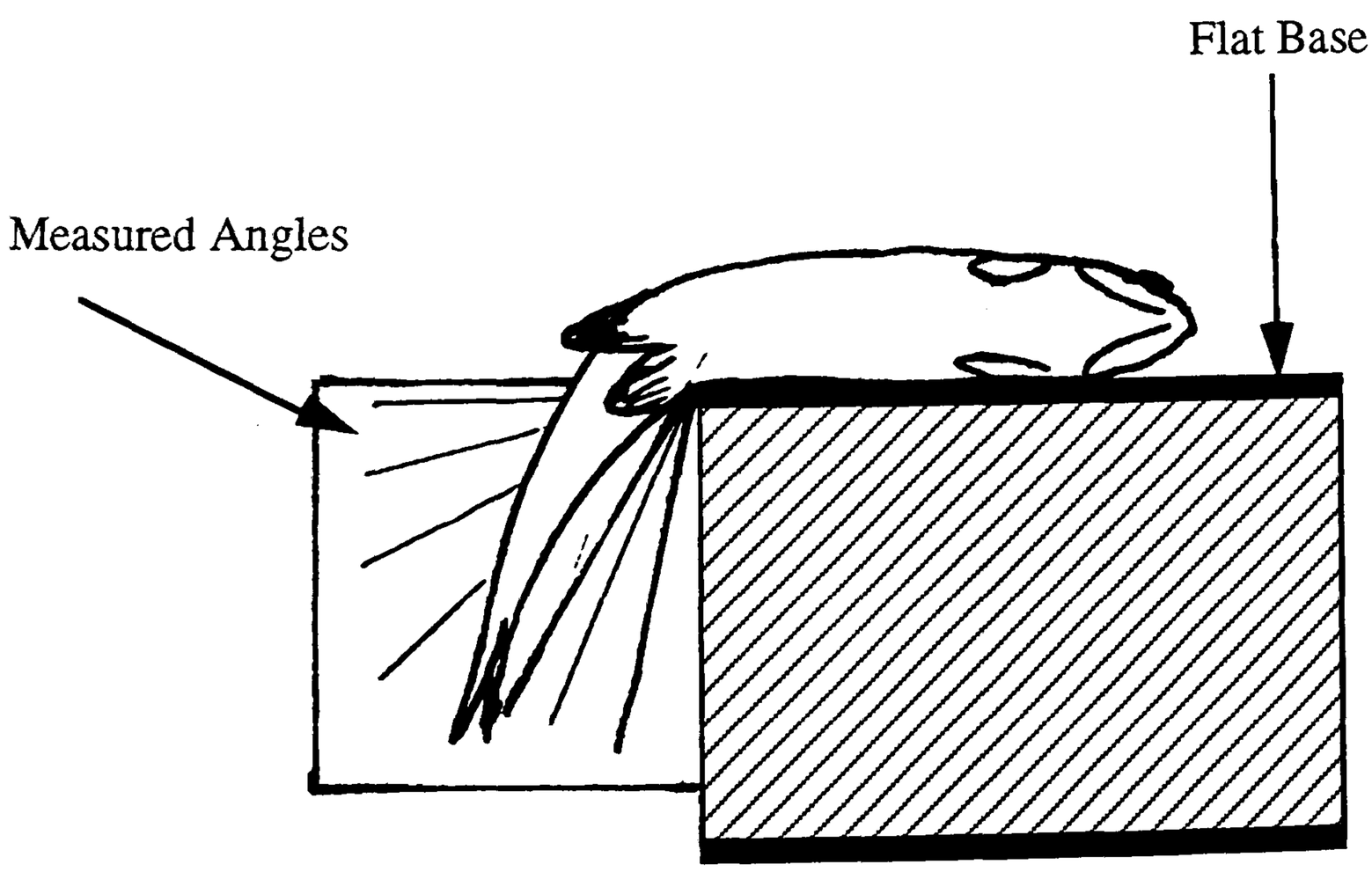

Figure 4.2.3: Apparatus for determining the degree of rigor in the fish.

4.2.3 Experiment 3

After analysis of the trout experiments, it was decided that the experiment should be repeated on Atlantic salmon. The aim of this experiment was to compare different current commercial practices with AQUI-S™ for behavioural and flesh quality responses. The results would give information to farmers on the effects of the slaughter methods on flesh quality and so help them to choose the optimum fish slaughter method. The slaughter methods chosen were AQUI-S™ and carbon dioxide anaesthesia, percussion stun and crowding followed by percussion stunning.

This experiment was carried out at the Marine Harvest McConnel FTU on Loch Eil, Scotland. The fish used were all Atlantic salmon grilse which were just starting to show the first signs of maturation — i.e. they were just starting to change colour from silver to red. At this stage of maturation there is generally little change in flesh quality. The fish were all from the same stock, having smolted after one year in freshwater and being grown in the sea for 26 months prior to the trial. Prior to this trial the fish had all been in the same trials cage and had received a pigmented commercial diet.

4.2.3.1 Slaughter

One hundred and twenty Atlantic salmon grilse were split between three trials cages (5m x 5m x 5m), in such a way that two cages had thirty fish in and the third had sixty fish. The fish were starved for four days before transfer and were starved for a further three days after. During the last three days they were left undisturbed to recover from the handling stress.

i) Crowding Period and Percussion Stun

On the day of slaughter, the nets of one of the cages with thirty fish in were lifted up to crowd the fish for a period of four hours. Every hour the nets were drawn up further to crowd the fish more. This was done every fifteen minutes during the last hour, to try to simulate the conditions in a commercial crowd prior to slaughter. After four hours of crowding, the fish were netted out by hand, two at a time, hit on the head with a priest and exsanguinated by slitting all of the gill arches on one side of the head. The fish were then individually tagged and placed in a bin of ice slurry to bleed out.

ii) Carbon Dioxide Anaesthesia

A bin of sea water, approximately $1m^3$ in volume, was placed beside the cage with sixty fish in. Carbon dioxide gas was slowly bubbled into the water in the bin through fine gas diffusers until the water was saturated with the gas. This was determined by the pH — the water being saturated when the pH fell below 4.5 (SSGA guidelines, Anon, 1995). When the pH had reached this level, the nets were raised in the cage and thirty fish were netted out by hand and placed into the bin. The gas supply was then turned off and a lid placed on the bin. After the last fish had been in the bin for six minutes the lid was lifted off and the fish checked for responses to touch. When no responses were shown the fish were lifted out, individually tagged and all the gill arches on one side of the head cut to allow exsanguination. The fish were then transferred to the bin of ice slurry to bleed out.

iii) Percussion Stun

The remaining thirty fish from that cage were then netted out by hand, two at a time, hit on the head with a priest to stun them, individually tagged and the gill arches on one side of the head slit. The fish were placed in the bin of ice slurry.

iv) AQUI-S™ Anaesthesia

The final cage of thirty fish was enclosed by a tarpaulin after the nets had been raised slightly to reduce the volume without stressing the fish. The volume of water enclosed was calculated and AQUI-S™ added to the water to result in a concentration of 17ppm. A garden sprayer was used to added the AQUI-S™ already diluted with sea water in the ratio of 1:10. This allowed the anaesthetic to mix thoroughly with the water in the cage.

After forty minutes all the fish in the cage had succumbed to the anaesthetic and were netted out by hand. Each fish was hit on the head with a priest to stun it, individually tagged and the gills on one side of the head slit to allow exsanguination. The fish were then placed in the bin of ice slurry to allow them to bleed out.

The timing of the different slaughter processes was controlled so that all the fish were killed within forty minutes. They were all placed into the same bin of ice slurry which was thoroughly mixed so that the rate of cooling was approximately the same for all groups. This was carried out to eliminate any effects of temperature on the experiment.

4.2.3.2 Processing and Measurements

After slaughter the fish were taken in the bins of ice slurry to a processing plant as rapidly as possible. Here the fish were individually weighed and eviscerated. Twenty fish from each slaughter group were selected at random, filleted and the temperature, pH and colour measured as in the previous experiments. The colour at the initial measurement point was measured with the chroma meter and a Roche colour card. Measurements were taken at 5.75, 11.5, 20.25, 24.5, 36, 48.5, 54.5, 72, 95, and 168 hours after slaughter.

i) Roche Colour Card

The colour card scoring was performed by two assessors, with the fillets illuminated in a light box. This was a box shielded from external light in which the fillets were evenly illuminated, reproducible for each batch. A second colour card measurement was taken at the final point, but unfortunately the light box was not available at this point (see section 4.3.3.7). Different assessors also had to be used at this point, therefore no comparisons could be made between the first and the last measurement points.

After each series of measurements the fillets were placed in polystyrene boxes, ten fillets per box. In the bottom of each box there was a layer of ice covered by a thin plastic sheet on which the fillets were placed so that the area of flesh to be measured on each fish was exposed. A second layer of plastic covered the fillets and more ice was placed on top. The lid was then placed on the box and the box kept in a chiller at 4°C. The ice was replaced as required.

ii) Lipid Analysis

After the final measurement a sample of flesh was cut from each fillet. The sample was taken from below the dorsal fin and the skin, bones and the very top and bottom of the dorsal and belly lipid depots respectively trimmed off as described for the Scottish Quality Salmon cut (Anon, 1995). Each sample was then blended using a Moulinette blender (Moulinex, UK) and a subsample analysed for lipid and moisture content using the CEM rapid lipid analysis technique as in chapter 3.

iii) Rigor

The remaining ten fish from each group were kept unfilleted and were used for rigor measurements as in the previous experiment. Rigor measurements were carried out at 3.75, 11.5, 20.25, 24.5, 36, 48.5, 54.5, 72, and 95 hours after slaughter. After measuring the fish were placed in polystyrene fish boxes, seven fish per box. In the bottom of each box there was a layer of ice, on which the fish were placed, ventral side down, so that no water collected in the visceral cavity. A layer of ice was placed on top of the fish and the lid placed on the box. The boxes were then stored in a chiller at 4°C.

4.2.3.3 Data Analyses

The three experiments each produced large data sets due to the large number of measurements taken from each fillet. The data from each experiment was treated separately as the fish were slaughtered at different times of the year and so may have differed in pigment levels and other flesh properties. Further, the levels of stress and exercise may have differed between experiments.

The mean and standard errors of the temperature, chroma meter measurements and the rigor angles were determined for each slaughter group in the three experiments. This allowed graphs of the changes of the temperature, lightness, angle of hue, chroma and state of rigor to be plotted.

The pH values for each fillet were converted to hydrogen ion concentrations using the formula:

$$\text{Hydrogen ion concentration} = 10^{(-\text{pH})}$$

This converted the results into linear variables, allowing the mean and standard errors for each treatment to be determined. The mean value for each slaughter treatment was then reconverted to pH using the formula:

$$\text{pH} = -\log(\text{hydrogen ion concentration})$$

Graphs of the changes in pH could be plotted from these data. The standard errors for each point were added and subtracted from the mean hydrogen ion concentration. These values were then converted to pH values as above and were plotted on the graphs as the estimates of standard errors. This was to allow some estimation of the variation at each point to be visualised.

The mean Roche colour card scores given by the assessors for each individual fillet were determined. The mean and standard error of the mean for each treatment was found and plotted graphically.

The results were analysed statistically using a one factor ANOVA (Stat View, Abacus Concepts Inc., Berkeley, USA) at each time. This allowed the effect of the treatments on hydrogen ion concentration, colour and rigor to be determined.

The results of the gaping analyses had to be treated differently as they were non-parametric data. In the first and third experiments, as there were more than two groups involved, a difference was searched for using the Kruskal- Wallis non-parametric test (Stat View) with the null hypothesis that there was no difference. Following the rejection of the null hypothesis the treatments were compared pairwise using the Mann-Whitney U-test (Stat View). For the second experiment there was no need to analyse the results with the Kruskal-Wallis non-parametric test first, as there were only two groups, and so just the Mann-Whitney U-test was used.

4.3 Results

4.3.1 Experiment 1: Small Rainbow Trout

4.3.1.1 Observations

i) Anaesthetised

The application of the anaesthetic 2-phenoxyethanol was carried out with great care, with the anaesthetic being added to the water in 5ml doses. This was because a violent reaction is shown towards rapid anaesthesia by fish. This effect has been observed with all the anaesthetics used regularly for fish in Great Britain — including MS222 and benzocaine solutions. Previous observations during anaesthesia showed that 2-phenoxyethanol resulted in the least reaction (pers. obs.). It was important to minimise this reaction as it obviously increased muscle activity.

When the anaesthetised fish were hit on the head with the priest muscle tremors were observed along their flanks. These were also observed on unanaesthetised fish and were taken as a sign of an effective stun — a secondary sign of an effective stun was the loss of eye movement when the fish was rotated. The degree of muscle tremor at this point may have slightly affected the final experimental results due to the amount of muscle activity involved.

ii) Netted then Killed

When the unanaesthetised fish were netted from tank B the capture was as rapid as possible to minimise the stress on the netted group of fish. Reducing the water level helped to speed up the capture process, but it was inevitable that fish were chased round the tank before being caught. Once in the net and removed from water the fish

showed violent escape behaviour until hit on the head with the priest. All the fish were successfully stunned by the first blow with the priest- indicated by the muscle tremors and subsequent loss of eye movement. Previous experience showed that the failure of the first blow to achieve a stun resulted in violent escape behaviour by the fish, which were obviously highly stressed at this point, as would be expected.

iii) Electro-stimulated

During electro-stimulation violent muscle activity was observed. For approximately the first twenty seconds the fish vibrated along its length as all the muscles contracted and relaxed rapidly. This resulted in a great deal of movement, which broke the electrical contacts during stimulation of two of the fish. In these cases the electrodes were replaced and the stimulation continued.

After approximately thirty seconds the violent movements ceased completely and only the jaw, eyes and fins could be seen to twitch. Eye and jaw movements ceased after the first minute, but the fins continued to twitch throughout the entire period of stimulation. At the end of the stimulation the whole carcass visibly relaxed as the current was switched off. After the degree of activity exhibited by the body muscles and the subsequent cessation of movement it could be assumed that the muscle cells were close to, or had achieved, exhaustion.

iv) Post-slaughter Storage

During the post-slaughter storage some seepage of blood and exudate occurred from the fillets. Blood was especially noticeable in the anaesthetised group during the first hour of storage. Loss of exudate occurred from all fillets throughout the experiment. Before each measurement was taken the exudate was wiped off with a piece of absorbent paper to ensure that the surface condition of each fillet was the same.

4.3.1.2 Fillet Temperature

The core temperature of the fillets was measured in the same place each time. The probe was inserted to the same depth, which was approximately half the thickness of the fillet. This was to avoid coming close to the skin which was closer to the layer of ice.

The mean temperatures for each group are shown in table 4.3.1. There were a lot of significant differences between groups, but the actual temperature differences were very small. This underlined the importance of mixing up groups during storage to eliminate the storage conditions as factors which affected the results.

Table 4.3.1: Mean fillet core temperature. Standard errors are given in parentheses. Means in the same row with different superscript letters are significantly different ($p<0.05$).

Anaesthetised		Netted		Electro-stimulated	
Time (h)	Temp. (°C)	Time (h)	Temp. (°C)	Time (h)	Temp. (°C)
0	13.4 (0.04)^{a}	0	14.1 (0.18)^{b}	0	14.1 (0.12)^{b}
1.2	2.6 (0.28)^{a}	1.2	2.6 (0.27)^{a}	1.3	2.4 (0.38)^{a}
2.4	1.0 (0.16)^{a}	2.2	1.8 (0.19)^{b}	2.5	1.2 (0.16)^{a}
5.6	0.2 (0.10)^{a}	5.4	1.5 (0.11)^{b}	5.8	1.5 (0.07)^{b}
8.4	0.0 (0.04)^{a}	9.2	-0.2 (0.04)^{b}	9.1	0.0 (0.05)^{a}
21.2	0.2 (0.06)^{a}	22.0	0.1 (0.09)^{ab}	21.9	0.0 (0.03)^{a}
32.8	-0.1 (0.05)^{a}	33.8	-0.4 (0.03)^{b}	33.6	-0.2 (0.02)^{a}
44.8	-0.1 (0.03)^{a}	45.7	-0.6 (0.02)^{b}	45.5	-0.3 (0.03)^{c}
57.2	0.1 (0.02)^{a}	58.2	-0.5 (0.01)^{b}	58.0	0.0 (0.02)^{c}
69.3	0.4 (0.04)^{a}	70.2	0.5 (0.07)^{b}	70.0	0.3 (0.04)^{a}

The results are displayed graphically in figure 4.3.1. All groups of fillets cooled at approximately the same rate after they were placed in the ice. A difference in the temperature of the groups can be seen at approximately 5 hours after slaughter. This was probably caused by the two boxes with the netted and the electro-stimulated

fillets running low on ice, as this was immediately after transport from the fish farm to Langford. All the boxes were topped with fresh ice immediately after this reading and this cooled the fillets down to the same level as the anaesthetised fillets.

By approximately 10 hours after slaughter the fillets had reached a core temperature of just below 0°C. They remained at approximately this temperature throughout the rest of the storage period.

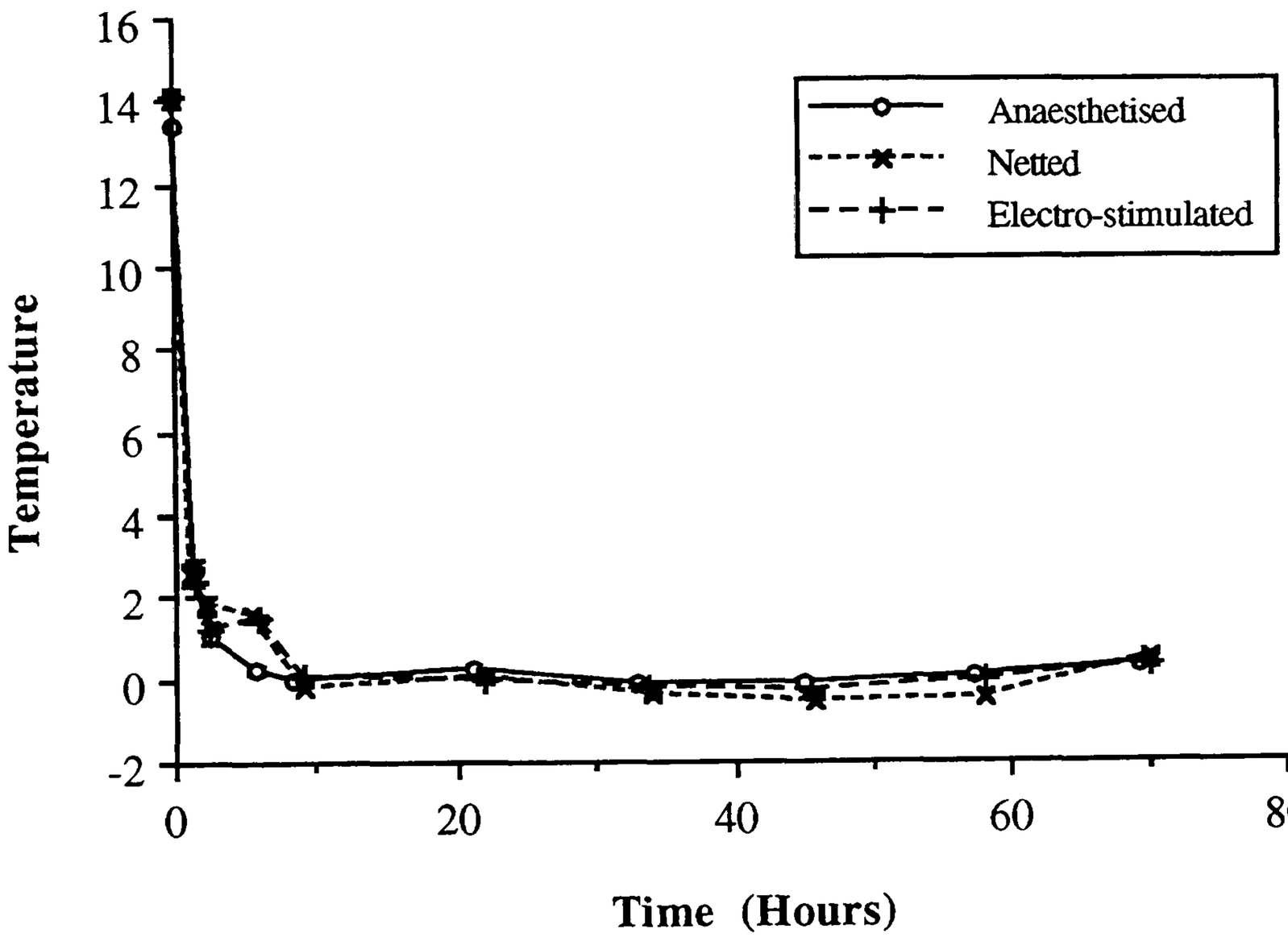

Figure 4.3.1 Temperature cooling curve of the fillets. Standard errors plotted about the mean for each group.

4.3.1.3 Fillet pH

The portable pH meter was re-calibrated for every measurement point and checked against standards regularly during the measurements. This ensured that the probe was accurately measuring the pH of the fillets.

The presence of muscle exudate caused great fluctuations in the pH readings as would be expected. Therefore great care was taken to wipe all the exudate from the surface of the fillets before the measurements were taken. During the measurements care was taken to hold the probe still as this affected the measurements by allowing exudate from the muscle to flow into the hole made by the probe.

The mean pH values and the results of the one factor ANOVA are shown in table 4.3.2. Significant differences were found between the anaesthetised group and the other two groups throughout the experiment ($p<0.05$). Significant differences also were found between the netted and electro-stimulated groups until approximately 22 hours after slaughter ($p<0.05$). From that point no differences were observed between these two groups.

Figure 4.3.2 shows that the pH of the anaesthetised group remained high for about 20 hours after slaughter. It then dropped from above pH 7 to approximately pH 6.6 where it remained for the rest of the storage period. The pH of the netted fillets fell more rapidly, dropping below pH 7 after about 3 hours of storage. However, the pH did not reach below 6.6 until about 20 hours after slaughter. It then remained close to this level for the remainder of the storage period, although it was still below the pH of the anaesthetised fillets. The fillets of the electro-stimulated group fell instantly to below pH 7. The pH was below 6.6 after about two and a half hours and continued to fall to a minimum of 6.38 ± 0.015 after 9.07 hours. The pH then rose slightly to about

6.5 for the rest of the storage period, matching the pH of the netted group approximately 48 hours after slaughter.

Table 4.3.2 Mean flesh pH. Figures in parentheses are standard errors. Means in rows with different superscript letters are significantly different ($p<0.05$).

Anaesthetised		Netted		Electro-stimulated	
Time (h)	pH	Time (h)	pH	Time (h)	pH
0.00	7.76 (0.306)[a]	0.00	7.12 (0.044)[b]	0.00	6.69 (0.027)[c]
1.22	7.50 (0.129)[a]	1.18	7.28 (0.073)[a]	1.28	6.77 (0.025)[b]
2.38	7.38 (0.061)[a]	2.17	6.93 (0.087)[b]	2.48	6.59 (0.024)[c]
5.62	7.34 (0.067)[a]	5.38	6.84 (0.074)[b]	5.85	6.51 (0.015)[c]
8.42	7.41 (0.029)[a]	9.22	6.86 (0.051)[b]	9.07	6.38 (0.015)[c]
21.18	7.31 (0.052)[a]	22.02	6.59 (0.018)[b]	21.87	6.50 (0.016)[c]
44.83	6.63 (0.010)[a]	45.67	6.54 (0.013)[b]	45.53	6.50 (0.014)[b]
57.22	6.57 (0.010)[a]	58.17	6.47 (0.010)[b]	57.97	6.48 (0.019)[b]
69.28	6.65 (0.011)[a]	70.18	6.60 (0.012)[b]	70.03	6.58 (0.13)[b]

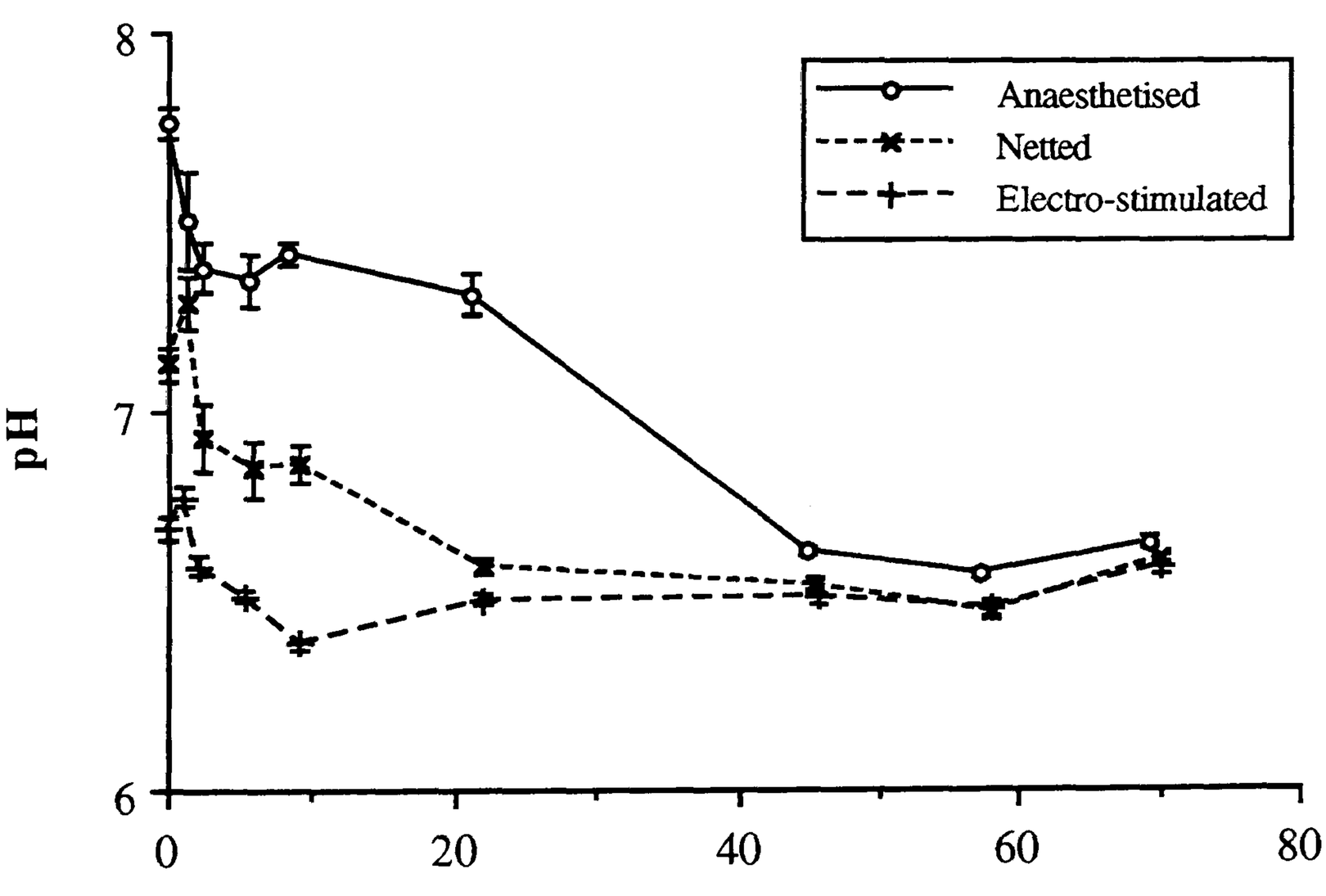

Figure 4.3.2 Changes in mean pH (± s.e.m.) with time.

4.3.1.4 Chroma Meter Readings

The chroma meter readings were affected by the position of the head of the meter and the surface conditions of the fillet. Care was therefore taken to wipe the surface of the fillet with dry absorbent paper immediately prior to the measurements. This removed any blood seepage and collection of exudate from the surface which may have altered the readings. Care was also taken to place the head of the meter in the same region of the fillet each time, whilst the head was moved slightly for each of the triplicate measurements at each time point to allow for the myosepta, as has been described.

The lightness, L*, means of the individual fillets were compared using a one-factor ANOVA (table 4.3.3). There were significant differences between the anaesthetised group and the other two groups throughout most of the experiment. Few differences were observed between the netted and electro-stimulated groups.

Table 4.3.3 Mean fillet lightness, L* values — figures in parentheses are standard errors. Means in rows with different letters in superscript are significantly different ($p<0.05$).

Anaesthetised		Netted		Electro-stimulated	
Time (h)	L*	Time (h)	L*	Time (h)	L*
0.0	38.98 (0.691)^{a}	0.0	42.10 (0.716)^{b}	0.0	40.74 (0.957)^{ab}
1.2	36.34 (0.809)^{a}	1.2	40.71 (0.386)^{b}	1.3	38.85 (1.104)^{b}
2.4	36.72 (1.051)^{a}	2.2	39.41 (0.464)^{b}	2.5	37.58 (0.758)^{ab}
5.6	36.11 (0.639)^{a}	5.4	38.75 (0.916)^{a}	5.8	38.32 (1.185)^{a}
8.4	35.25 (0.657)^{a}	9.2	37.19 (0.588)^{b}	9.1	38.41 (0.883)^{b}
21.2	35.61 (0.720)^{a}	22.0	39.34 (0.601)^{b}	21.9	40.10 (0.849)^{b}
32.8	34.76 (0.594)^{a}	33.8	39.43 (0.870)^{b}	33.6	41.73 (0.984)^{b}
44.8	36.88 (0.581)^{a}	45.7	40.10 (0.593)^{b}	45.5	41.28 (0.774)^{b}
57.2	38.77 (0.683)^{a}	58.2	41.68 (0.544)^{b}	58.0	40.71 (1.113)^{ab}
69.3	38.25 (0.685)^{a}	70.2	41.49 (0.561)^{b}	70.0	41.75 (0.900)^{b}
74.7	39.02 (0.378)^{a}	76.0	42.54 (0.585)^{b}	75.6	43.00 (0.588)^{b}

Figure 4.3.3 shows that the L* values of all groups dropped slightly after slaughter and then increased from about 9 hours after slaughter in the stressed groups and after about 33 hours in the anaesthetised group. The lower L* values of the anaesthetised group throughout the trial are highlighted by the figure as is the similarity of the values for the two stressed groups. This indicates that the anaesthetised fish had darker flesh than the more active groups.

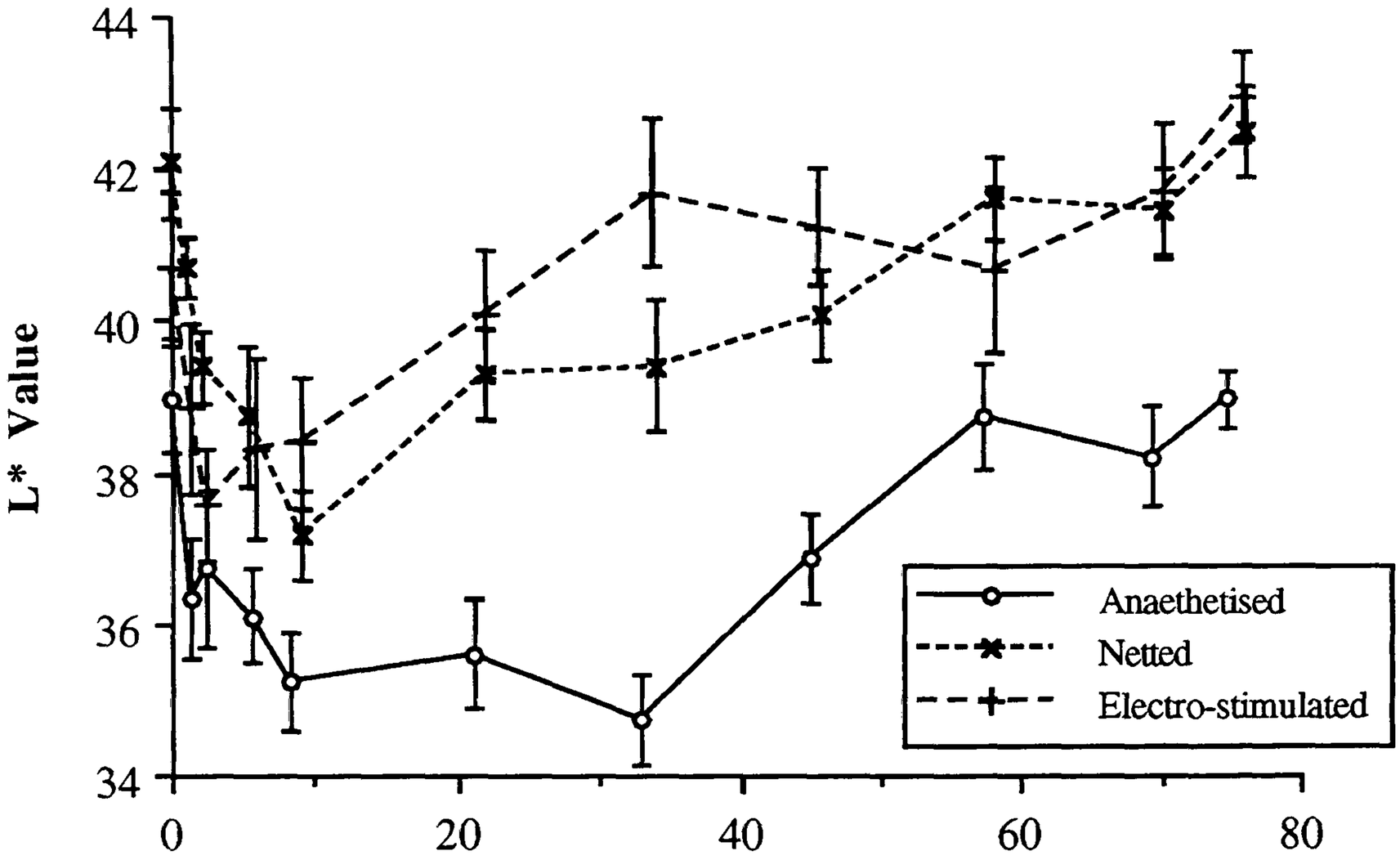

Figure 4.3.3 Change in L* value (± s.e.m.) with time post-slaughter.

The mean angle of hue for the groups of fillets is shown in table 4.3.4. There were many significant differences between the anaesthetised group and the other two groups ($p<0.05$). A significant difference between the netted and the electro-stimulated groups was only recorded after approximately 9 hours of storage ($p<0.05$).

Table 4.3.4 Mean fillet hue (± s.e.m.). Means in rows with different letters in superscript are significantly different ($p<0.05$).

Anaesthetised		Netted		Electro-stimulated	
Time (h)	Hue	Time (h)	Hue	Time (h)	Hue
0.0	44.08 (0.733)^{a}	0.0	47.84 (0.907)^{b}	0.0	46.43 (1.726)^{ab}
1.2	39.62 (1.381)^{a}	1.2	45.75 (0.528)^{b}	1.3	45.78 (0.601)^{b}
2.4	38.50 (1.376)^{a}	2.2	44.40 (0.700)^{b}	2.5	43.40 (0.780)^{b}
5.6	39.20 (0.752)^{a}	5.4	42.56 (0.810)^{b}	5.8	42.78 (1.340)^{a}
8.4	38.17 (0.679)^{a}	9.2	40.92 (0.459)^{b}	9.1	43.55 (0.615)^{c}
21.2	37.51 (1.013)^{a}	22.0	43.93 (0.467)^{b}	21.9	45.22 (0.718)^{b}
32.8	38.70 (0.617)^{a}	33.8	43.96 (0.500)^{b}	33.6	44.73 (0.692)^{b}
44.8	39.39 (0.612)^{a}	45.7	45.07 (0.337)^{b}	45.5	45.78 (0.674)^{b}
57.2	43.37 (0.649)^{a}	58.2	46.59 (0.587)^{b}	58.0	44.65 (1.133)^{ab}
69.3	42.33 (0.388)^{a}	70.2	47.17 (1.291)^{b}	70.0	44.88 (1.010)^{ab}
74.7	43.28 (0.541)^{a}	76.0	45.92 (0.430)^{b}	75.6	46.40 (0.710)^{b}

Figure 4.3.4 shows the changes of mean fillet angle of hue throughout the storage period. This highlights the significantly lower angle of hue of the anaesthetised group throughout most of the storage period. It can also be seen that the angle of hue of all groups dropped during the first 9 hours of storage. The anaesthetised group remained at the lower angle of hue until more than 44 hours after slaughter. However, the other groups showed an increase in the angle of hue by 22 hours after slaughter and remained at this higher level until the end of the storage period. The anaesthetised group showed a rise in the angle of hue after 44 hours, approaching the angle of hue shown by the other groups after 57 hours. However, the angle still remained significantly lower. This indicates that the colour of the anaesthetised fish was significantly closer to red (0°) than that of the fish in the more active groups, the colour of which was closer to yellow (90°).

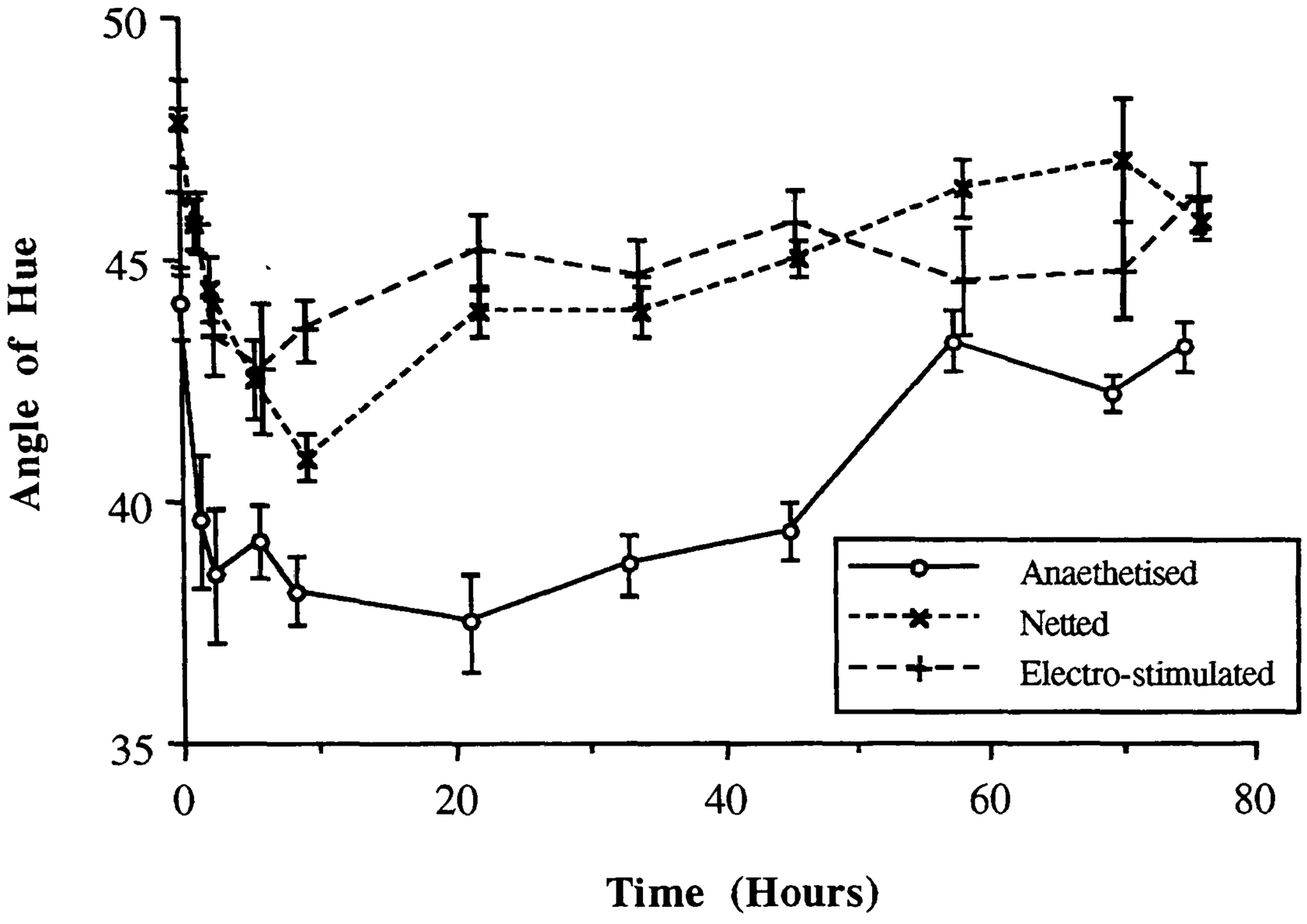

Figure 4.3.4 Change in the angle of hue (± s.e.m.) with time post-slaughter.

The chroma values were calculated for each group and are shown in table 4.3.5. The chroma of the anaesthetised group was significantly higher than that of the electro-stimulated group for the first 5 hours after slaughter. The chromas were then identical at about 9 hours after slaughter, before the chroma of the anaesthetised group dropped below that of the electro-stimulated group. The netted group showed chroma values in-between the anaesthetised and electro-stimulated groups.

Table 4.3.5 Mean fillet chroma- standard errors are in parentheses. Means in rows with different letters in superscript are significantly different ($p<0.05$).

Anaesthetised		Netted		Electro-stimulated	
Time (h)	Chroma	Time (h)	Chroma	Time (h)	Chroma
0.0	24.96 (0.725)^{a}	0.0	24.98 (0.625)^{a}	0.0	20.67 (0.878)^{b}
1.2	24.79 (0.681)^{a}	1.2	24.99 (0.473)^{a}	1.3	20.82 (0.920)^{b}
2.4	24.95 (0.935)^{a}	2.2	24.58 (0.684)^{a}	2.5	20.60 (0.884)^{b}
5.6	23.25 (0.911)^{a}	5.4	24.33 (1.168)^{a}	5.8	22.55 (1.324)^{a}
8.4	23.46 (1.127)^{a}	9.2	22.33 (0.685)^{a}	9.1	23.47 (1.228)^{a}
21.2	18.62 (1.066)^{a}	22.0	20.61 (0.767)^{ab}	21.9	22.57 (0.852)^{b}
32.8	19.71 (1.036)^{a}	33.8	22.31 (0.990)^{a}	33.6	27.31 (1.176)^{b}
44.8	19.68 (1.296)^{a}	45.7	22.64 (1.010)^{ab}	45.5	24.47 (0.830)^{b}
57.2	21.04 (1.320)^{a}	58.2	23.34 (0.836)^{b}	58.0	24.62 (1.323)^{b}
69.3	20.25 (0.982)^{a}	70.2	22.90 (1.053)^{ab}	70.0	25.31 (0.914)^{b}
74.7	20.35 (0.840)^{a}	76.0	22.64 (0.830)^{ab}	75.6	23.65 (0.918)^{b}

Figure 4.3.5 clearly shows the changes in chroma values of the anaesthetised group relative to the electro-stimulated group. The anaesthetised group showed a drop of chroma value throughout the storage period, whilst the electro-stimulated group showed a rise before becoming steady after about 44 hours of storage. The intermediary values of the netted group can be clearly seen.

A higher chroma value indicates higher opacity. Therefore it can be seen that the anaesthetised fish had more translucent flesh than the more active fish.

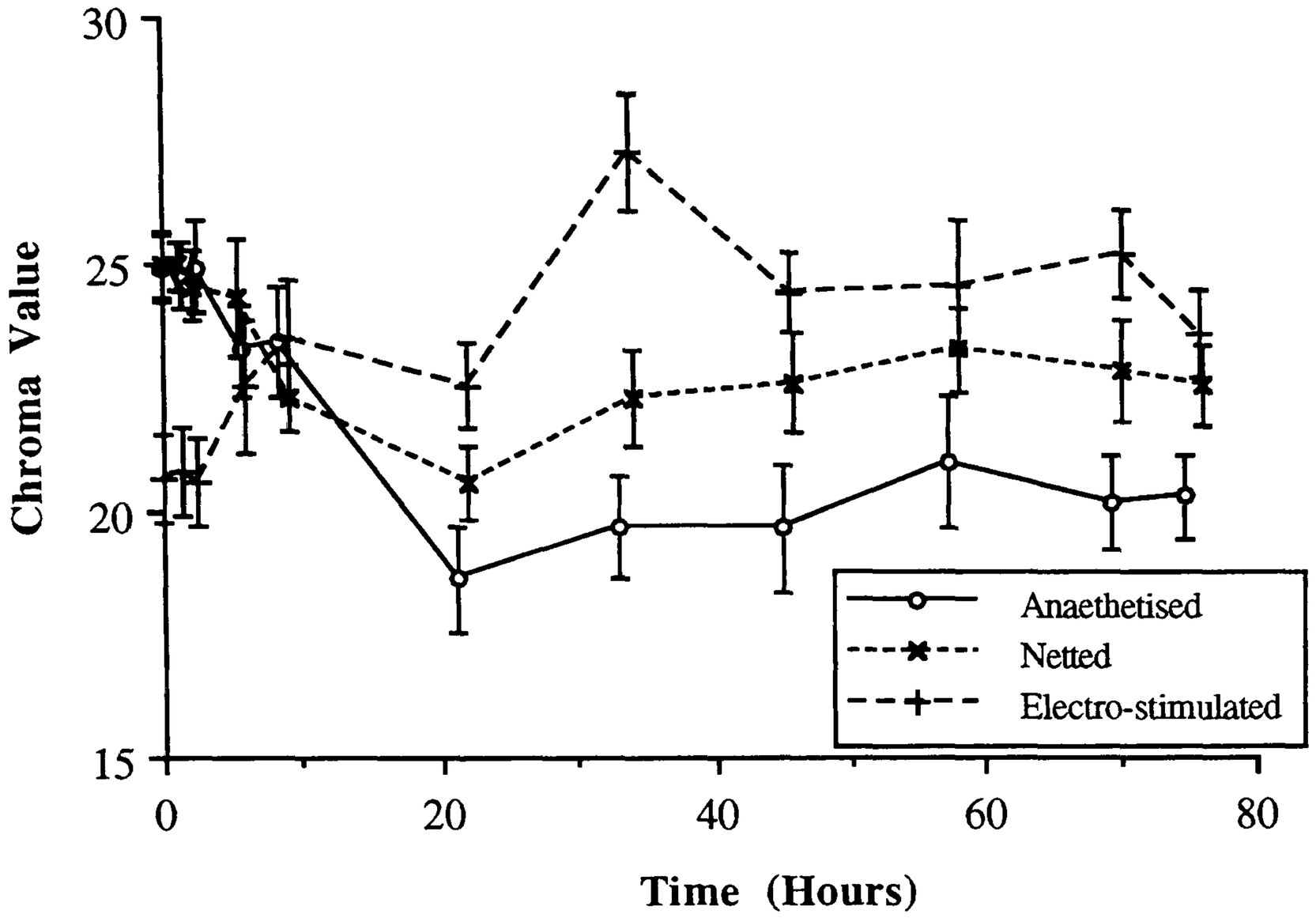

Figure 4.3.5 Change in mean chroma of the fillets (± s.e.m.) with time post-slaughter.

4.3.1.5 Roche Colour Card Scores

The five assessors were given a brief training session immediately prior to the assessment to refamiliarise themselves with the use of the card. Their scores for the fillets were then recorded.

The mean Roche colour card score was calculated for each fillet. All scores for each fillet were compared using regression analysis and the residuals checked for randomness. As there were no trends in the residuals, the scores from each assessor were compared using a two-factor ANOVA (StatView). The results are displayed in table 4.3.6.

Table 4.3.6 Results of a two-factor ANOVA on the colour scores, comparing the effect of treatments and assessor.

Source	df	F-test	p Value
Slaughter Treatment	2	4.393	$p < 0.01$
Assessor	4	7.225	$p < 0.001$
Treatment x Assessor	8	3.034	$p < 0.01$

The mean scores from each group were plotted in figure 4.3.6. The colour score of the fillets was found to decrease with increasing activity. Thus the anaesthetised group had the highest score (15.0 ± 0.18) and the electro-stimulated the lowest (14.2 ± 0.23).

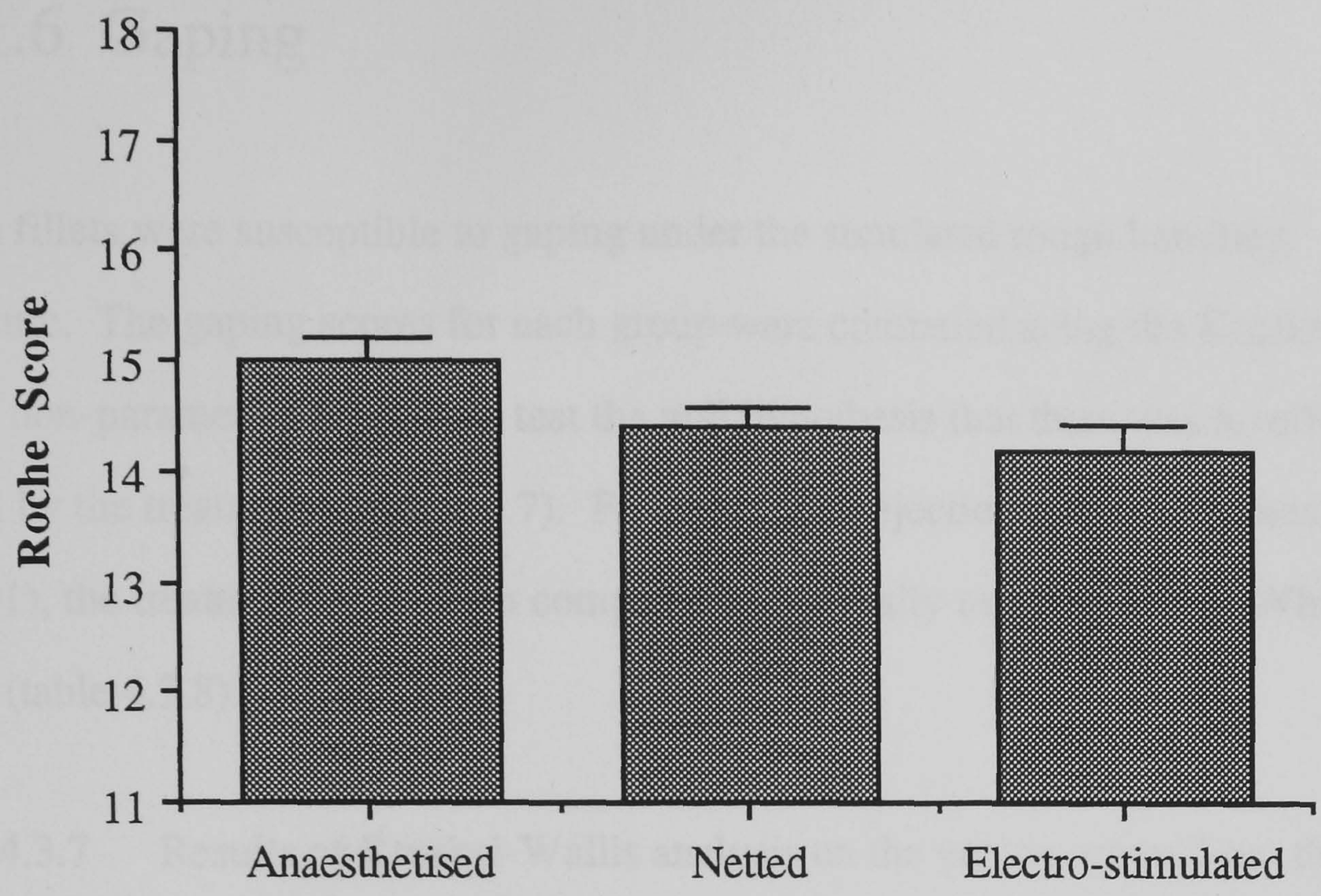

Figure 4.3.6 Mean Roche colour card score for each treatment (+ s.e.m.) after 70 hours of storage on ice.

4.3.1.6 Gaping

All the fillets were susceptible to gaping under the simulated rough handling procedure. The gaping scores for each group were compared using the Kruskal-Wallis non-parametric analysis to test the null hypothesis that there was no effect caused by the treatment (table 4.3.7). Following the rejection of this hypothesis ($p<0.01$), the treatments were then compared individually using the Mann-Whitney U-test (table 4.3.8).

Table 4.3.7 Results of Kruskal-Wallis analysis on the gaping scores from the three treatments.

df	2
H corrected for ties	12.92
p Value	0.002

Table 4.3.8 Results of Mann-Whitney U Test on the gaping scores of each treatment. p Values are for Z corrected for ties.

	U Value	p Value
Anaesthetised vs Netted	17	$p < 0.01$
Anaesthetised vs Electrocuted	9.5	$p < 0.01$
Netted vs Electrocuted	34	NS

Figure 4.3.7 shows the mean gaping scores for each group. This allows the visualisation of the differences between the groups, highlighting the lower gaping score of the anaesthetised group compared to the netted and electro-stimulated groups.

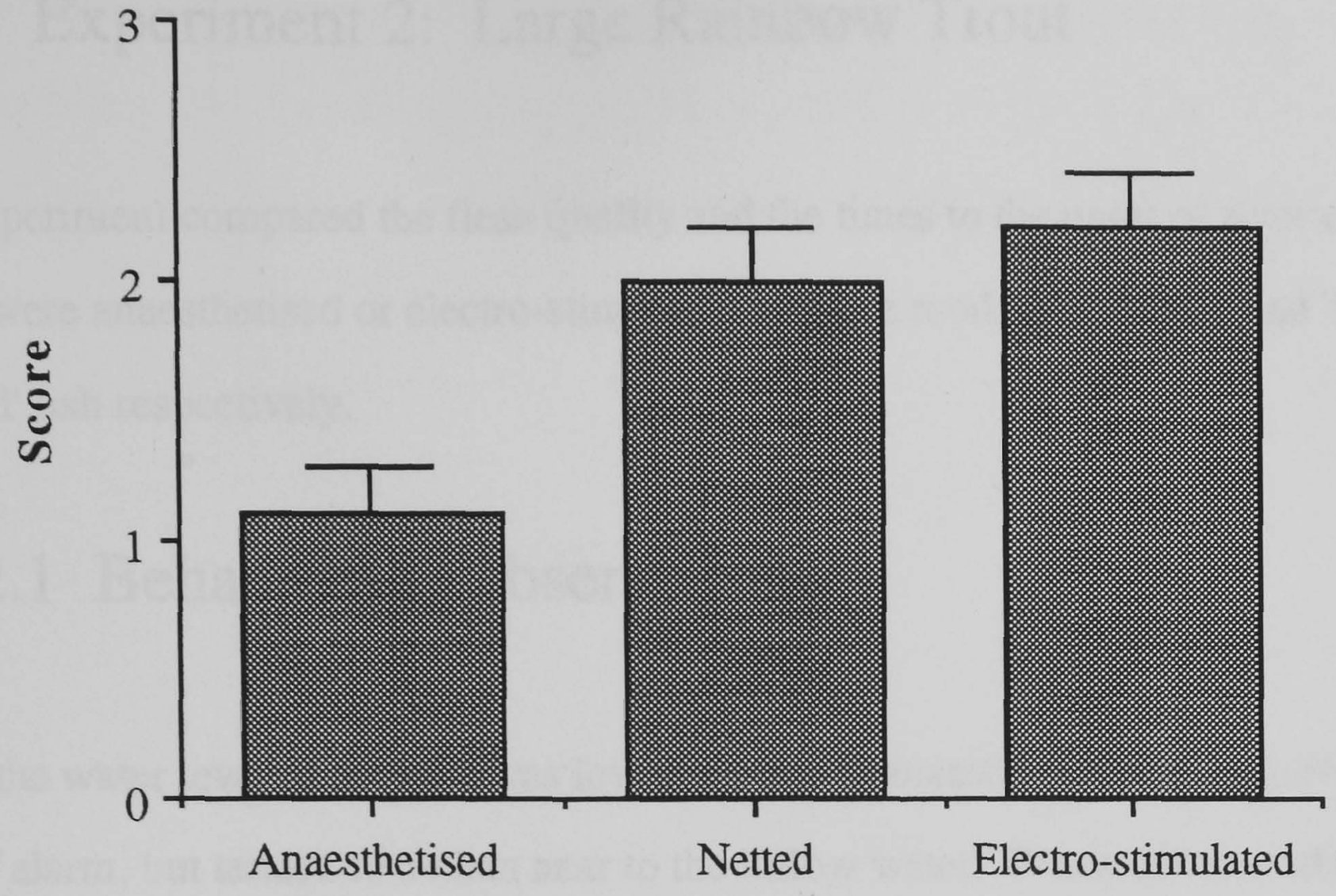

Figure 4.3.7 Mean gaping score for each treatment (+ s.e.m.) after 70 hours of storage on ice.

4.3.2 Experiment 2: Large Rainbow Trout

This experiment compared the flesh quality and the times to the onset of rigor of fish which were anaesthetised or electro-stimulated to make models of 'rested' and 'highly stressed' fish respectively.

4.3.2.1 Behavioural Observations

When the water level in the tank was lowered prior to anaesthesia the fish showed no sign of alarm, but tended to collect near to the inflow water. When the anaesthetic was added to the inflow the fish showed no aversive reaction; in fact some of the fish actually swam right up to the end of the sprayer unit, where the concentration of the anaesthetic was greatest.

Ten minutes after the application of the anaesthetic the fish started to lose co-ordination of their movements. Twenty minutes after application all the fish were upside down, but some swimming movements were still observed. After thirty minutes all movements had stopped apart from opercular activity. When the fish were netted out no escape behaviour was observed in the tank or after the fish was removed from the water. The only movement occurred when the fish were hit with the priest, causing the characteristic muscle tremors along the flank.

The anaesthetic had no apparent effect on the degree of muscle activity resulting from electro-stimulation. The amount of activity shown by the stimulated fish was approximately the same as for the first experiment when the fish were unanaesthetised. Thus the two groups of high and low activity fish were achieved.

After filleting no obvious blemishes, such as haemorrhages, were observed in either group of fillets. During storage some blood seeped from the fillets, but no difference was observed between the groups as occurred in experiment 1.

4.3.2.2 Fillet Temperature

The core temperature of the fillets was measured in the same place each time as for experiment 1. The mean results and the standard errors of the means are shown in table 4.3.9. The core temperature of the fillets dropped rapidly after they were placed in between the layers of ice (figure 4.3.8). Two hours after slaughter the fillets were almost at 2°C and after six hours they were below 0°C.

After the initial measurements there were no differences between the core temperatures of the two groups of fillets. The difference observed at the initial measurement point was probably because the electro-stimulated fish were out of the water longer than the anaesthetised fish, and so had warmed up in the air. Another factor in the increased temperature could have been the large amount of muscle activity displayed by the electro-stimulated fish which would have released energy as heat.

Table 4.3.9: Fillet core temperature post-slaughter.

Time (Hours)	Anaesthetised Temperature (°C)		Electro-stimulated Temp. (°C)	
	Mean	s.e.m.	Mean	s.e.m.
0	12.7	0.33	13.4	0.19
2	2.1	0.25	2.1	0.17
4	0.9	0.11	0.7	0.03
6	-0.4	0.06	-0.5	0.02
8	-0.3	0.05	-0.4	0.00
10	-0.4	0.02	-0.5	0.02
12	-0.5	0.02	-0.6	0.02
20	-0.5	0.02	-0.6	0.01
24	-0.3	0.07	-0.2	0.04
30	-0.4	0.04	-0.5	0.02
34	-0.4	0.02	-0.5	0.01
48	-0.5	0.02	-0.5	0.02
56	-0.3	0.02	-0.3	0.02
72	-0.5	0.02	-0.5	0.02
96	-0.4	0.04	-0.5	0.03

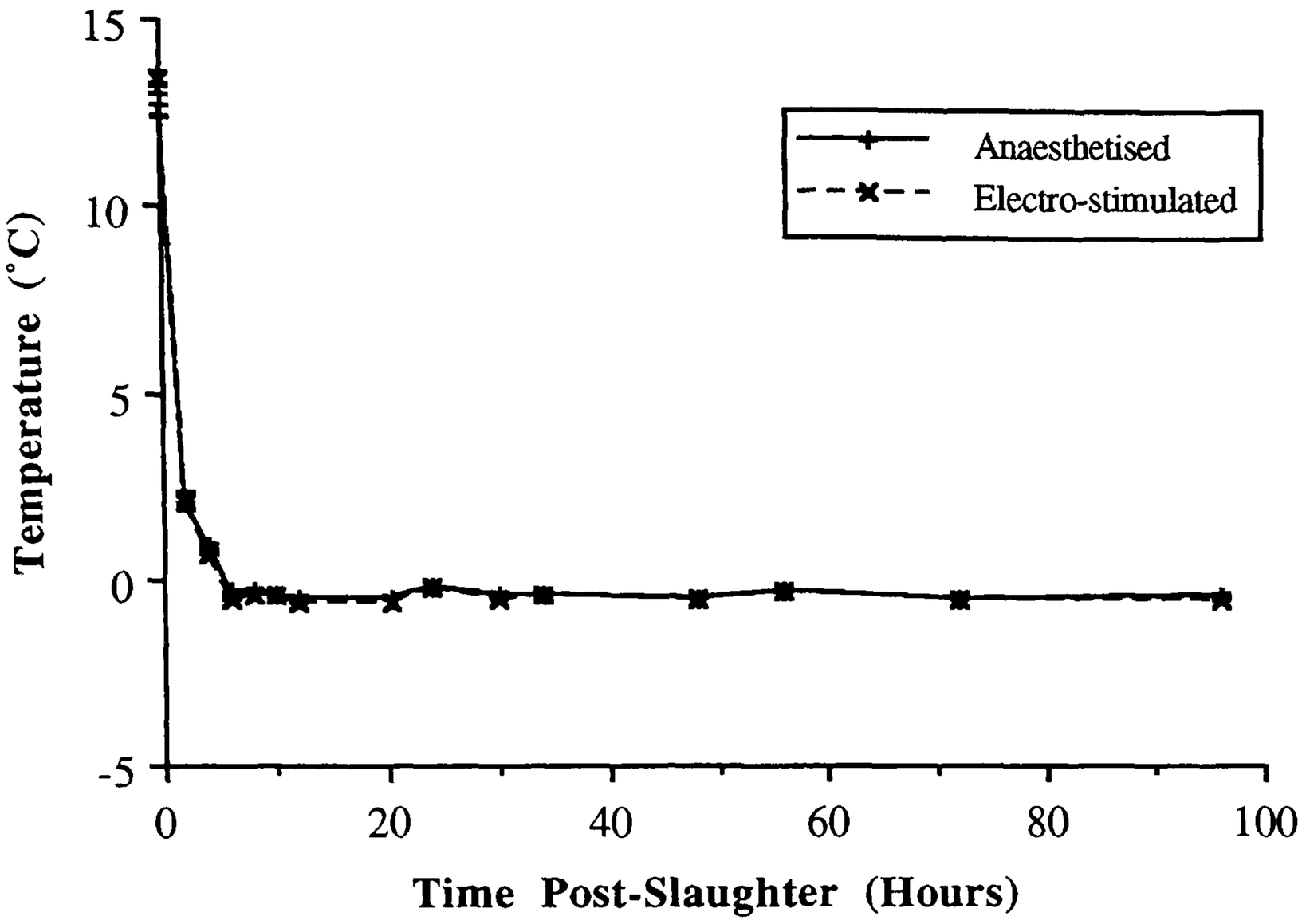

Figure 4.3.8: Change in mean fillet core temperature with time (± s.e.m.).

4.3.2.3 Fillet pH

The pH was measured as for experiment 1, with the same precautions being taken to minimise the effect of exudate on the pH readings. Table 4.3.10 shows the mean pH and standard errors of the means of the two groups of fillets. The results of the one factor ANOVAs carried out on the hydrogen ion concentrations are also shown in the table. These show that there was a significant difference between the hydrogen ion concentrations and thus the pH of the two groups of fillets until 72 hours after slaughter.

Figure 4.3.9 shows the change in mean pH of the fillets over time. The pH of the anaesthetised group dropped slowly over the first eight hours post-slaughter. The rate then appeared to increase until 34 hours post-slaughter when the fillets had almost attained their lowest pH. By 48 hours post-slaughter the pH of the fillets had stabilised at approximately pH 6.48.

The pH of the electro-stimulated fillets dropped very rapidly reaching the lowest pH 12 hours after slaughter. The pH then rose very slightly over the next 24 hours of storage from the lowest value of 6.37 ± 0.016 to stabilise at approximately pH 6.46.

Table 4.3.10: Mean fillet pH.

Time (Hours)	Anaesthetised Mean	s.e.m.	Electro-stimulated Mean	s.e.m.	Significance
0	7.48	0.050	6.81	0.037	<0.001
2	7.31	0.036	6.69	0.024	<0.001
4	7.38	0.042	6.77	0.041	<0.001
6	7.39	0.063	6.61	0.027	<0.001
8	7.19	0.124	6.54	0.024	<0.001
10	7.07	0.107	6.41	0.017	<0.001
12	7.10	0.107	6.37	0.016	<0.001
20	6.96	0.077	6.43	0.013	<0.001
24	6.69	0.067	6.40	0.008	<0.001
30	6.62	0.047	6.45	0.016	<0.01
34	6.52	0.014	6.46	0.013	<0.01
48	6.49	0.008	6.45	0.009	<0.01
56	6.49	0.011	6.46	0.008	<0.1
72	6.47	0.015	6.47	0.008	ns
96	6.48	0.011	6.46	0.008	ns

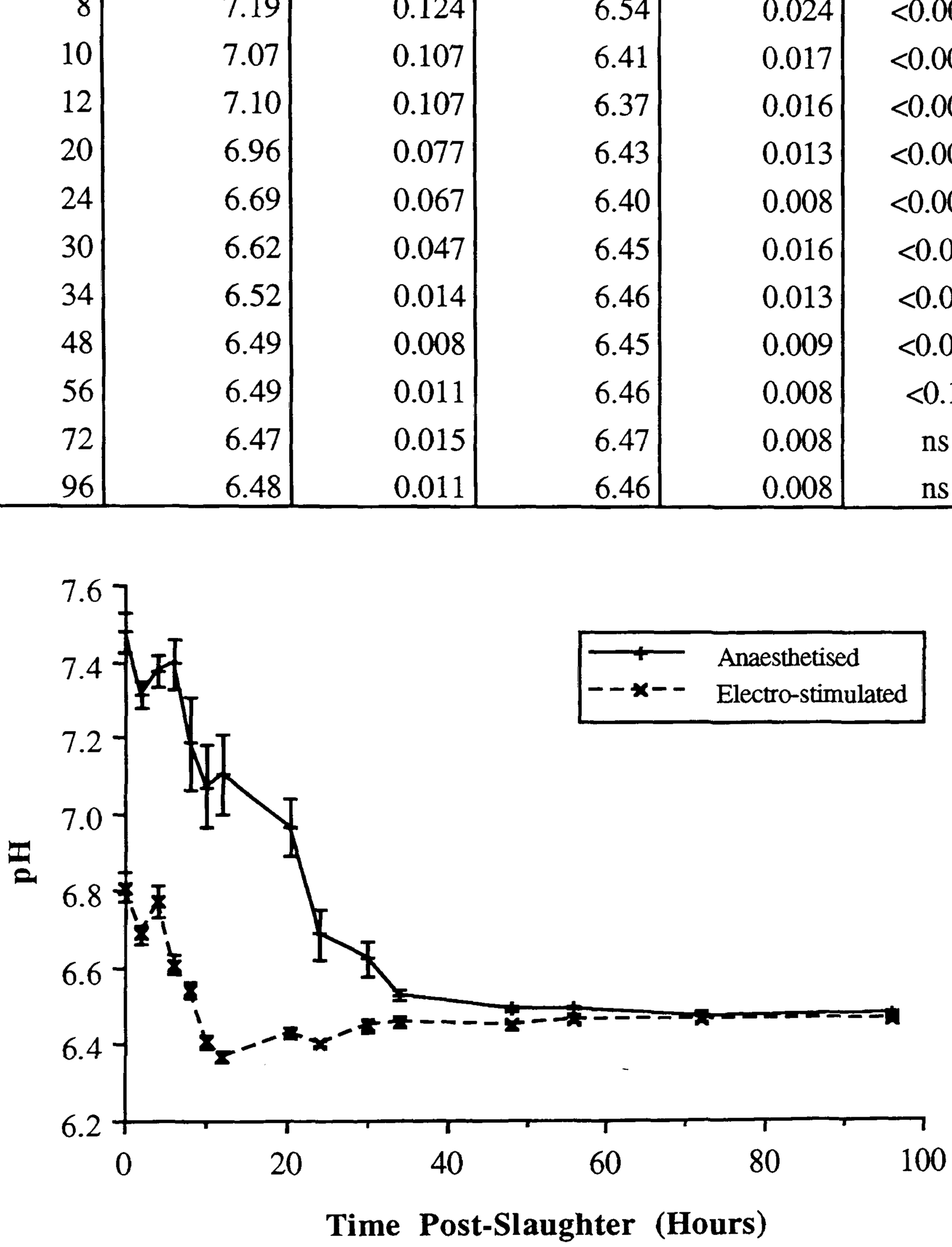

Figure 4.3.9: Changes in mean fillet pH (± s.e.m.) during post-slaughter storage.

4.3.2.4 Chroma Meter Measurements

The chroma meter measurements were taken using the same precautions as for experiment one. The mean L* values of the two groups of fillets are shown in table 4.3.11.

Table 4.3.11 Mean fillet L* values during storage.

Time (Hours)	Anaesthetised		Electro-stimulated		Significance
	Mean	s.e.m.	Mean	s.e.m.	
0	39.21	0.923	40.94	0.464	ns
2	37.35	0.843	39.88	0.446	<0.05
4	40.96	1.482	40.42	0.509	ns
6	41.71	1.478	41.95	0.809	ns
8	39.37	1.220	41.22	0.927	ns
10	40.30	0.938	42.97	0.949	<0.1
12	40.11	0.772	42.08	1.046	ns
20	40.49	0.934	42.24	0.904	ns
24	38.71	1.053	41.80	0.915	<0.05
30	40.99	1.072	43.33	0.712	<0.1
34	43.40	0.876	44.39	0.967	ns
48	43.39	1.854	44.02	0.678	ns
56	45.39	1.391	45.71	0.865	ns
72	42.45	1.460	45.25	0.797	ns
96	44.57	1.582	44.91	0.520	ns

There were few significant differences in fillet lightness between the groups during storage (table 4.3.11) — these occurred at 2 hours and 24 hours after slaughter, when the L* value of the anaesthetised group was significantly lower than that of the electro-stimulated group ($p<0.05$). At two times — 10 hours and 30 hours after slaughter — there were non-significant trends towards a difference ($p<0.1$). For the rest of the storage period there were no significant differences between the groups.

Figure 4.3.10 shows the changes in fillet lightness graphically. The lightness of both groups increased during storage by approximately 4 to 5 units. The figure also shows how closely together the two groups change.

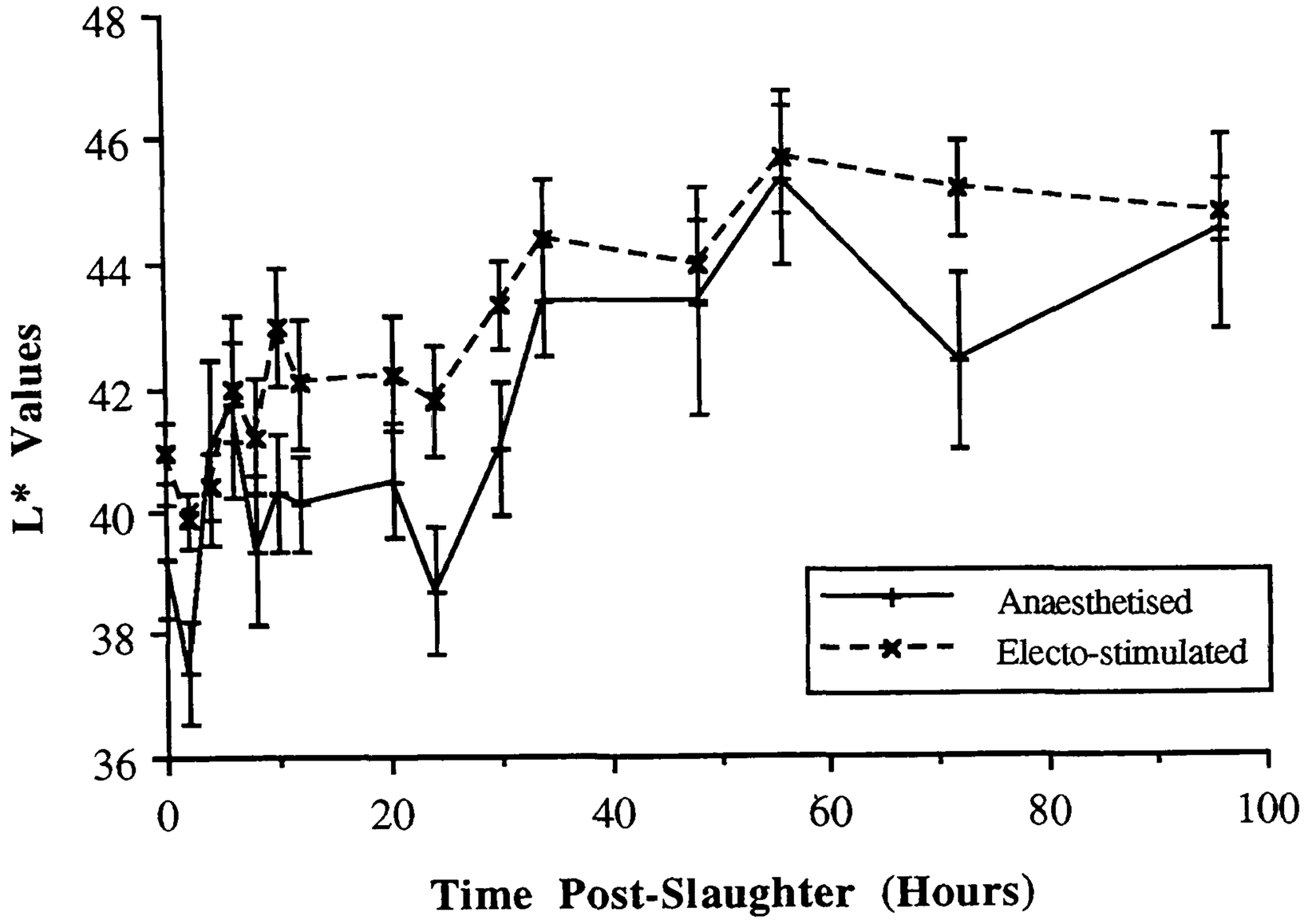

Figure 4.3.10 Changes in fillet lightness, L* (± s.e.m.) during storage,

Table 4.3.12 shows the mean values of fillet angle of hue during storage. From the table it can be seen that initially there were significant differences between the angle of hue of the two groups. The anaesthetised group had a significantly lower angle of hue than the electro-stimulated group for 30 hours after slaughter ($p<0.05$). Beyond this point there was still a trend towards there being a difference ($p<0.1$) but the difference was not significant.

Table 4.3.12: Mean fillet angle of hue.

Time	Anaesthetised (°)		Electro-stimulated (°)		Significance
(Hours)	Mean	s.e.m.	Mean	s.e.m.	
0	39.26	1.226	45.84	1.446	<0.01
2	37.82	1.343	45.45	1.272	<0.001
4	40.98	2.216	46.33	1.169	<0.05
6	41.12	1.741	46.88	1.033	<0.05
8	39.86	1.943	45.85	1.119	<0.05
10	41.35	1.375	46.39	1.294	<0.05
12	41.27	1.244	45.98	1.358	<0.05
20	42.28	1.537	46.64	1.258	<0.05
24	38.15	1.659	44.64	0.811	<0.01
30	40.17	1.676	45.28	0.729	<0.05
34	43.04	1.074	46.03	1.009	<0.1
48	41.74	2.040	45.59	0.709	<0.1
56	43.97	1.576	46.48	0.577	ns
72	43.08	1.688	46.55	0.579	<0.1
96	43.89	1.719	46.49	0.603	ns

Figure 4.3.11 shows the changes of the angle of hue graphically. The angle of hue of the electro-stimulated group stayed approximately the same throughout at about 46°. The angle of hue of the anaesthetised group started much lower at 39.3 ± 1.23°, but slowly increased during storage to 43.9 ± 1.72°. This showed that the anaesthetised fish had a redder flesh initially, but it became more yellow during storage.

Figure 4.3.11 also shows that the variation in hue, shown by the standard errors, of the

anaesthetised group was much larger than that for the electro-stimulated group after 48 hours of storage.

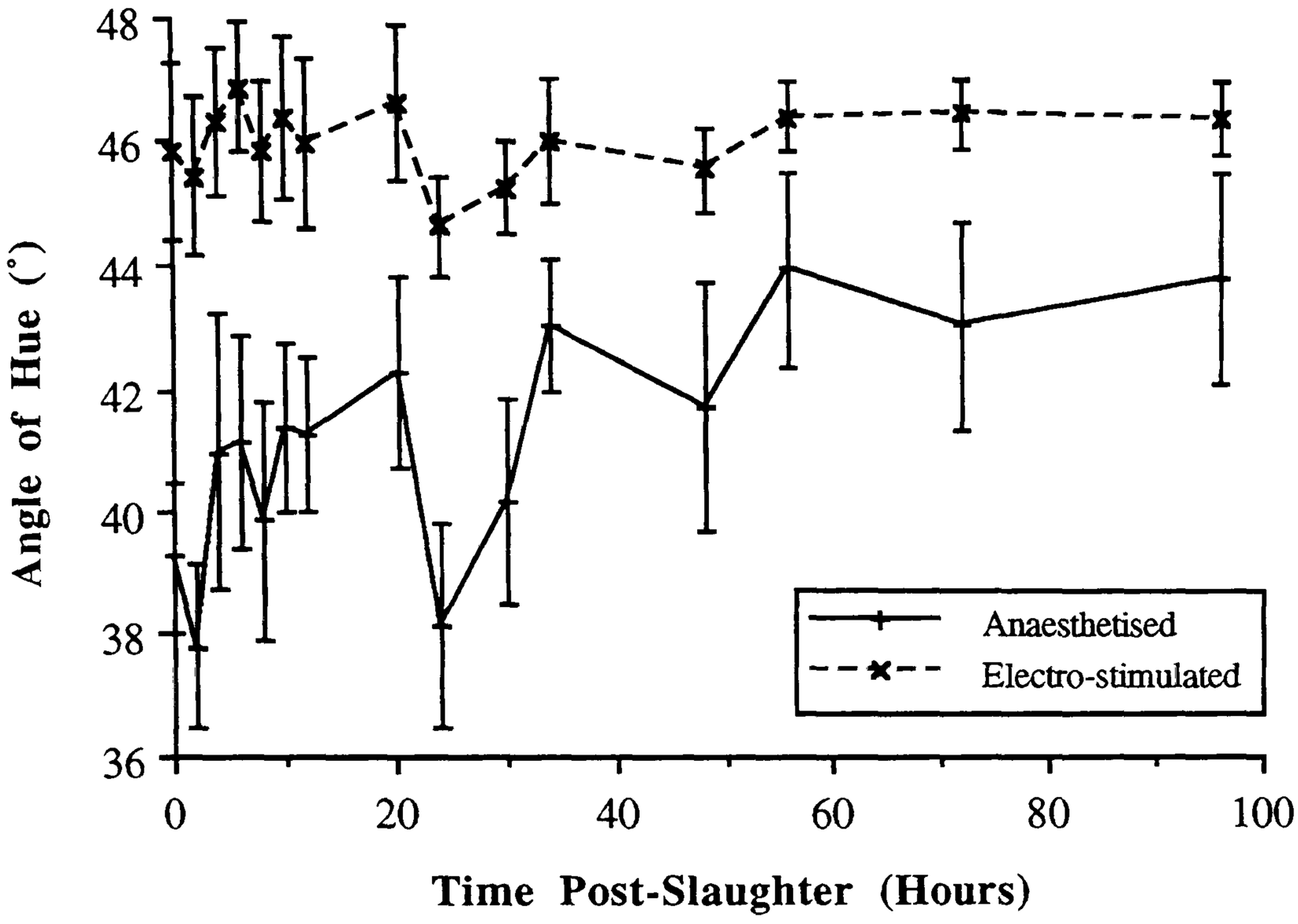

Figure 4.3.11 Changes in fillet angle of hue during post-slaughter storage (± s.e.m.).

The mean fillet chroma values are shown in table 4.3.13. There was only one point where there was a significant difference between the chroma values of the two groups — at 4 hours after slaughter, when the chroma of the electro-stimulated group was significantly lower ($p<0.05$). Throughout the rest of the storage period there were no differences in fillet chroma between the two groups.

Table 4.3.13 Mean fillet chroma values.

Time	Anaesthetised		Electro-stimulated		Significance
(Hours)	Mean	s.e.m.	Mean	s.e.m.	
0	25.74	0.708	24.05	1.361	ns
2	25.45	0.670	23.41	0.707	<0.1
4	25.89	0.758	22.58	0.717	<0.01
6	25.87	0.729	24.40	0.859	ns
8	24.88	0.616	23.23	0.971	ns
10	24.73	0.636	24.72	1.289	ns
12	25.42	0.699	24.92	0.866	ns
20	23.87	0.909	24.50	0.965	ns
24	23.90	0.471	24.77	0.853	ns
30	24.92	0.690	25.84	0.653	ns
34	26.37	0.659	26.59	0.928	ns
48	25.15	0.935	26.23	1.120	ns
56	27.03	0.941	26.58	0.723	ns
72	26.27	0.715	26.86	0.911	ns
96	26.06	1.105	26.35	0.725	ns

Figure 4.3.12 shows how the chroma of the two groups of fillets changed during storage after slaughter. The chroma of the anaesthetised group stayed approximately constant during the experiment at about 26 units after dipping slightly lower at about 20 hours after slaughter. The chroma of the electro-stimulated group started off lower at 24.0 ± 1.36 and rose for the first 30 hours after slaughter until it matched the chroma of the anaesthetised group. The chromas of the two groups then followed each other closely throughout the remainder of the study.

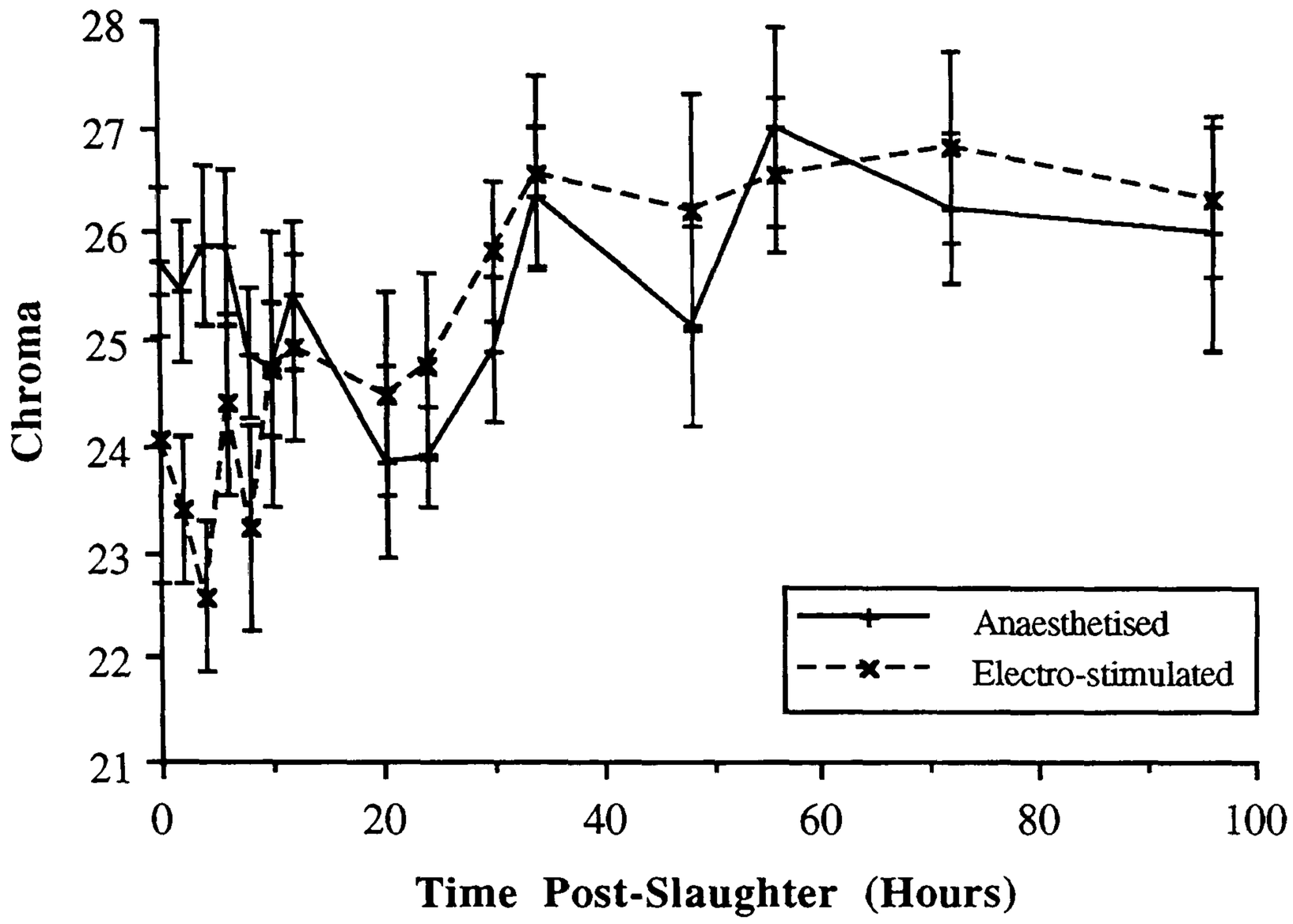

Figure 4.3.12 Changes in mean fillet chroma during post-slaughter storage (± s.e.m.).

4.3.2.5 Roche Colour Card Scores

The two assessors of colour with the Roche colour card had been chosen for their ability to score with approximately the same range and variation after investigation in the previous experiment (section 4.3.1). However, the results from the two different times cannot be compared as the lighting conditions were not controlled. Both sets of readings were conducted outside under north light, but the light intensity differed.

Table 4.3.14 shows the mean Roche colour card scores for both sets of fillets at the two measurement points after slaughter. The mean score was significantly greater for the anaesthetised group at both measurement points ($p<0.05$). The difference at both points in time was approximately one unit, as can be seen from figure 4.3.13.

Table 4.3.14 Mean Roche colour card scores of the fillets.

Time (Hours)	Anaesthetised		Electro-stimulated		Significance
	Mean	s.e.m.	Mean	s.e.m.	
24	16	0.25	15	0.21	<0.01
96	15	0.41	14	0.22	<0.05

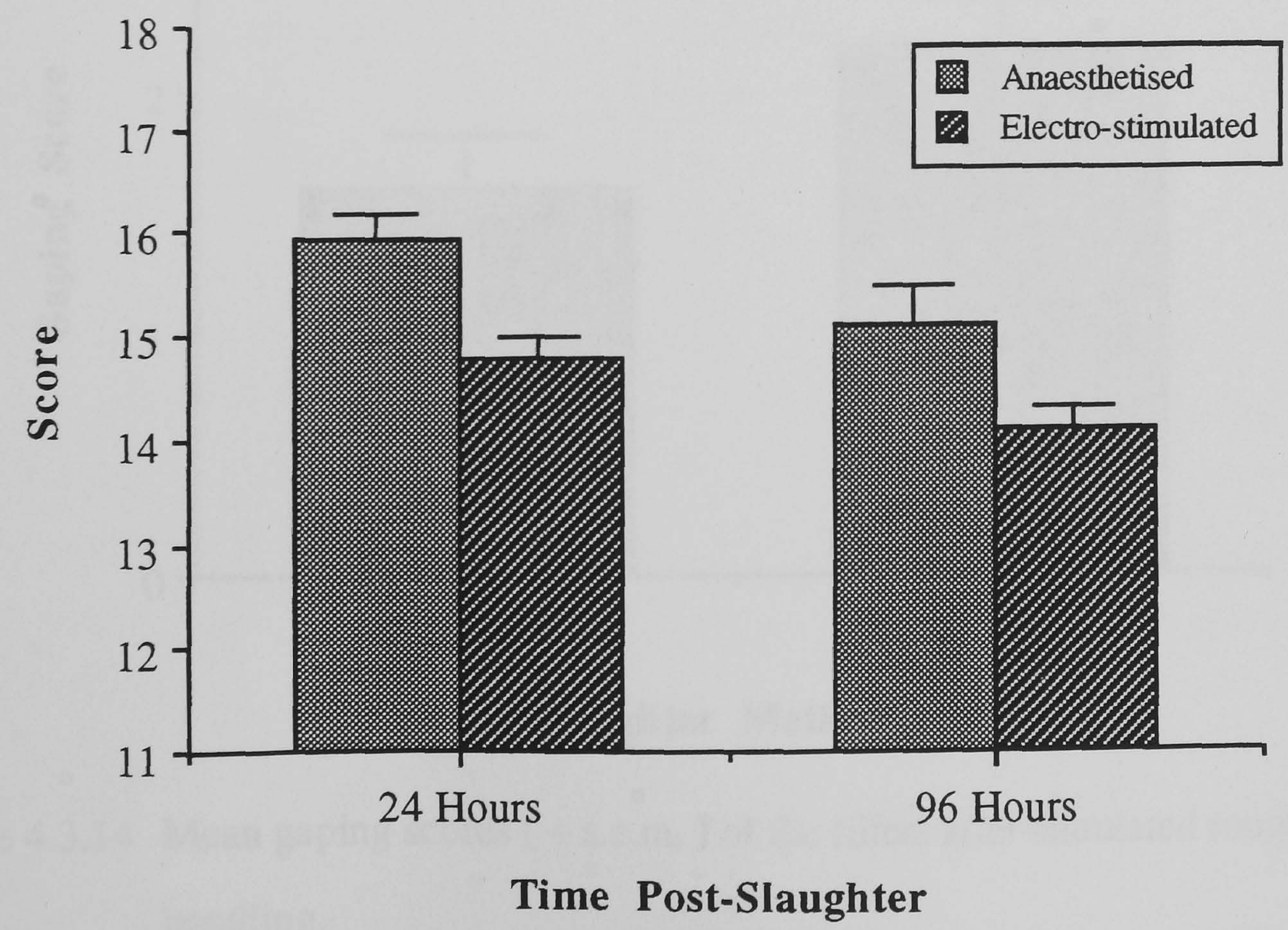

Figure 4.3.13 Differences in mean Roche colour card scores (+ s.e.m.).

4.3.2.6 Gaping

The fillets were all susceptible to gaping under the simulated rough handling procedure. The scores of the degree of gaping were determined according to the previously described four point system and the results of the Mann Whitney U-test on the scores are shown in table 4.3.15. There is a trend towards a difference in gaping scores, but the difference is not significant ($p<0.1$). Figure 4.3.14 shows the mean gaping scores for each group and from this it can be seen that the trend was towards the anaesthetised group having a lower gaping score than the electro-stimulated group.

Table 4.3.15 Results of a Mann Whitney U-test on the gaping scores of the fillets after a simulated rough handling procedure.

Time (Hours)	Mean Rank Anaesthetised	Electro-stim.	U Value	Significance
96	8.2	12.8	27	$p < 0.1$

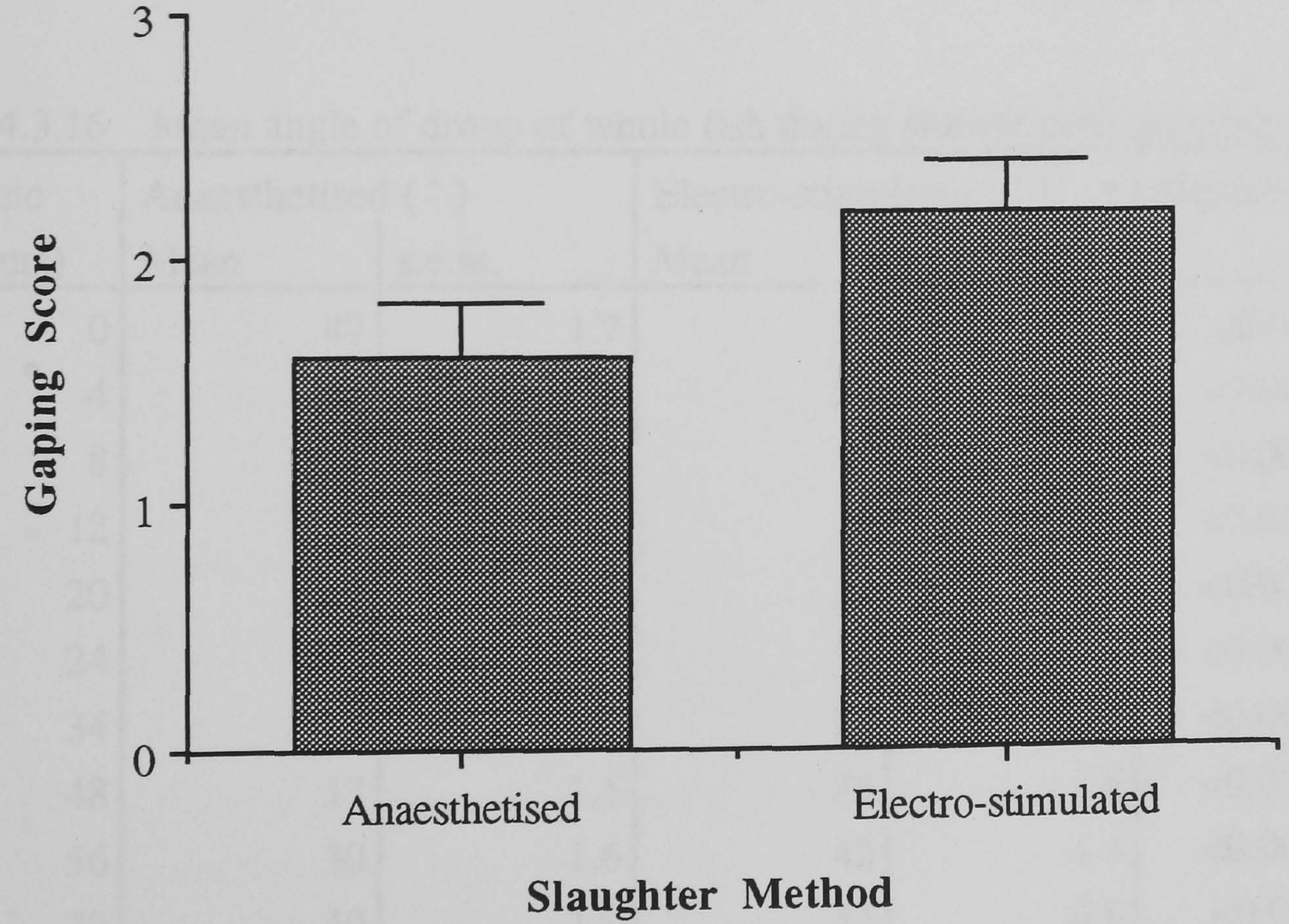

Figure 4.3.14 Mean gaping scores (+ s.e.m.) of the fillets after simulated rough handling.

4.3.2.7 Rigor

The angle of droop from the rear of the dorsal fin to the tip of the tail was straightforward to measure to an accuracy of 5°. Although this might not appear to be very accurate it was regarded as sufficient to follow the course of rigor, where three states can be recognised — 'out of rigor', in 'full rigor' and 'after resolution of rigor'. In view of this, a more accurate measurement, such as that described by Azam *et al.* (1990) was not required. By measuring both sides of the fish for droop and taking the mean, any predisposition towards bending to one side was allowed for. This was noticed to be especially important as rigor started to set in and later, as the fish started to become fixed in the position in which they lay.

Table 4.3.16 shows the changes in mean angles of droop during the experiment. Significant differences occurred between the angles of both groups throughout the experiment until 96 hours after slaughter when the mean angles were identical at 49.5°. The small standard errors show how small the variation within groups was.

Table 4.3.16 Mean angle of droop of whole fish during storage post-slaughter.

Time	Anaesthetised (°)		Electro-stimulated (°)		Significance
(Hours)	Mean	s.e.m.	Mean	s.e.m.	
0	47	1.7	56	1.7	<0.01
4	59	1.5	33	2.7	<0.001
8	51	2.1	9	1.5	<0.001
12	39	1.9	4	0.9	<0.001
20	39	2.7	13	1.1	<0.001
24	39	2.1	18	1.4	<0.001
34	11	1.6	24	1.7	<0.001
48	17	1.5	35	1.8	<0.001
56	30	1.6	42	1.4	<0.001
72	40	1.9	47	0.8	<0.01
96	49	1.3	49	0.7	ns

The changes in the angle of droop are shown in figure 4.3.15. The angle of droop of the anaesthetised fish stayed high — above 40°C — for the first 24 hours of storage. This was the period before the onset of rigor. The angle then dropped rapidly to below 20° at 34 hours after slaughter — at this point the fish could be considered to be in full rigor (Azam *et al.*, 1990). The angle then slowly rose to above 40° after 72 hours of storage, when the fish could again be considered out of rigor.

The angle of droop of the electro-stimulated fish dropped rapidly immediately after slaughter, reaching the lowest angle after 12 hours, although the fish could be considered to be in full rigor after 8 hours, as the angle was less than 20° (figure 4.3.15). By 24 hours after slaughter the angle was above 20° and rigor was starting to resolve. At 56 hours after storage the angle of droop was above 40° and the fish could be considered out of rigor.

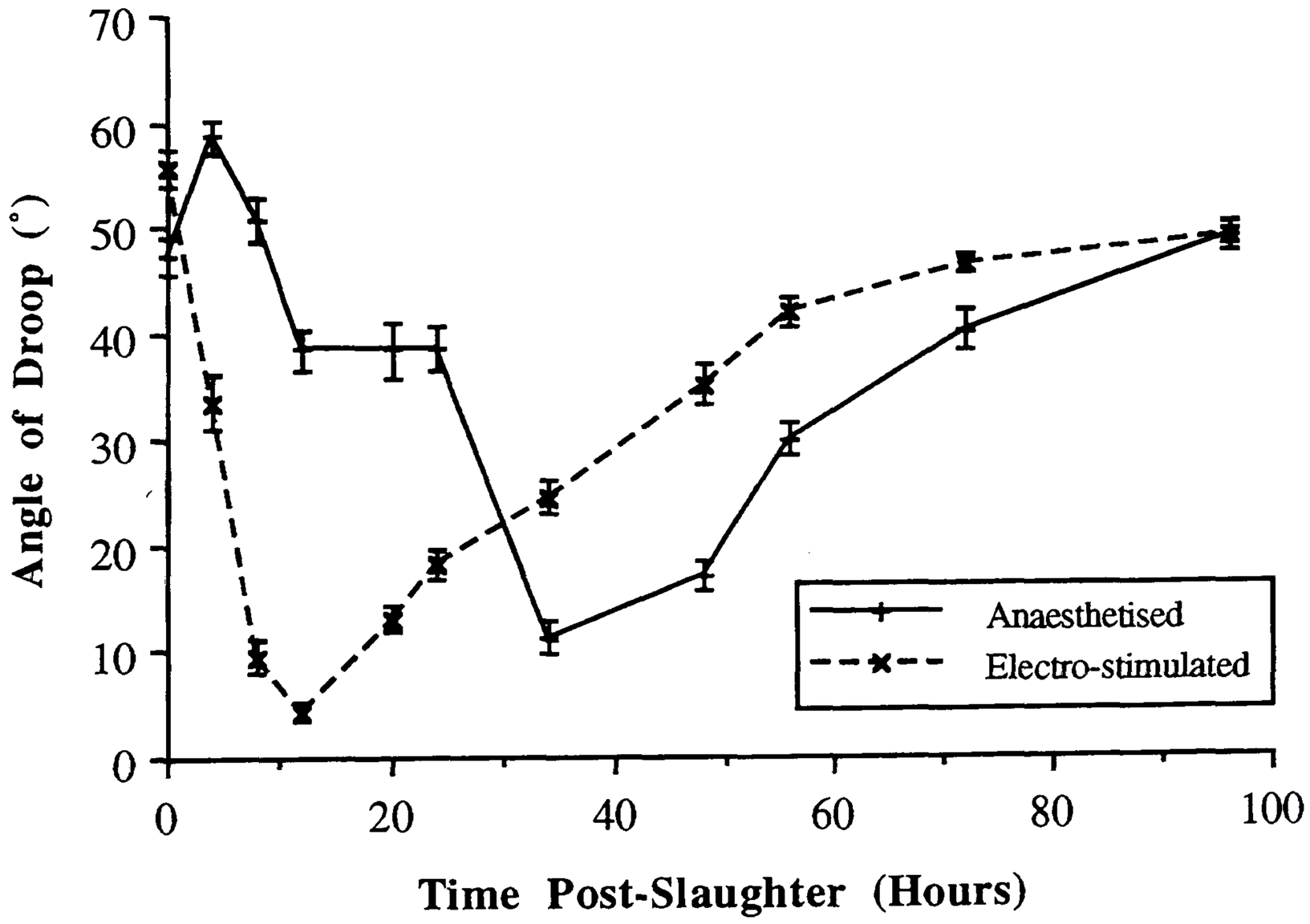

Figure 4.3.15 Onset and resolution of rigor during storage as determined by the angle of droop (± s.e.m.).

4.3.3 Experiment 3: Atlantic Salmon

4.3.3.1 Behavioural Observations

A video recording of the whole slaughter process was made. Observations on the behaviour of the fish made at the time and from the video are described below.

i) AQUI-S ™ Anaesthetised Fish

During the lifting of the nets and setting of the tarpaulin the fish showed no signs of disturbance. When the AQUI-S™ was added to water no aversive reactions were displayed, with the fish swimming right up to the end of the sprayer, where the AQUI-S™ was the most concentrated. Some fish even took the end of the sprayer into their mouths, though this did cause them to turn away.

After approximately ten minutes some fish were swimming very slowly and by fifteen minutes all the fish showed a degree of loss of co-ordination. By thirty minutes all fish were upside down at the surface of the water, with some fish still exhibiting an occasional tail flick. Forty minutes after the application of the anaesthetic all movement within the cage, apart from opercular beats, had stopped.

As the fish were lifted out of the cage in the hand nets, some showed signs of movement in response to exposure to air. This resulted in one or two tail flicks before the percussion stun could be applied. Such reactions occurred in about one third of the fish. On the application of the stun all fish displayed muscular tremors along the length of their body which lasted for a maximum of four seconds. After the gills were slit the blood ran freely before the fish were placed in the ice slurry, where exsanguination continued.

ii) Carbon Dioxide Anaesthetised Fish

The fish showed little disturbance during the slight crowding and the short period in the hand nets before being placed in the bin of carbon dioxide saturated seawater. The fish showed a few tail flicks in the nets, but this level of stress was insignificant compared to that displayed as soon as the fish entered the anaesthetic bath.

As soon as the fish entered the bath a very strong reaction was displayed. This involved violent thrashing in the water and rapid swimming about in the tank, with the fish often swimming into the sides of the tank. Other behaviour included rapid swimming with the head and gills out of the water, until the fish were unable to sustain this position and had to drop beneath the surface. A lid had to be placed on the bin to prevent the fish escaping. This behaviour lasted for about two minutes after entering the water. After this, movement reduced dramatically and quickly stopped.

Six minutes after the last fish was placed in the bin all movement had ceased. When the lid was lifted the surface of the water was covered with a thick foam from the activity of the fish. On lifting the fish out no reactions were shown to fin pinches, which indicated that the fish were fully anaesthetised (Anon, 1995). However, on gill slitting six fish still showed reactions to the cutting, with further tail flaps. After gill slitting the blood ran freely from the cuts until the fish were placed in the ice slurry.

iii) Percussion Stunned Fish

The fish showed little disturbance on crowding, which lasted for a maximum of ten minutes for the last fish taken out. During the netting procedure the fish were caught quickly, so little activity was displayed, even when the fish were brought into air before being stunned. In the net only one or two tail flicks were displayed before the stun could be applied.

One or two blows were required to stun the fish. Immediately after the final blow the fish exhibited a muscle tremor along the length of their body, which lasted a maximum of four seconds. If the initial blow was inaccurate and did not achieve a stun, the fish displayed aversive reactions which involved several tail flicks. However, the fish were restrained in the net and so the second blow could be quickly delivered.

After the final blow was delivered the fish were gill slit. The blood flowed freely from the cuts until the fish were placed in the ice slurry.

iv) Crowded Fish

When the nets were initially raised the fish showed a small degree of disturbance as the volume of the cage was decreased. The signs of disturbance increased with each successive lift of the nets and for the last thirty minutes of the crowd the fish were often at the surface displaying rapid tail flicks and some thrashing. This activity was not prolonged, but was repeated often.

When the fish were netted out of the water they displayed similar reactions to that shown by the percussion stunned fish — *i.e.* some tail flicks in the net and aversive reactions to a misplaced stun attempt. After gill slitting the blood ran freely until the fish were placed in the ice slurry.

4.3.3.2 Live Weight

The live weight of the fish is displayed in table 4.3.17. A one factor ANOVA showed that there was no significant differences between the weight of the fish in each group ($p<0.05$). Thus live weight could be ruled out as a factor affecting the results.

Table 4.3.17 Live weights of the fish in the slaughter groups.

Slaughter Method	Mean (kg)	s.e.m.
AQUI-S™	3.25	0.049
Carbon Dioxide Anaesthesia	3.26	0.510
Percussion Stun	3.19	0.046
Crowd and Percussion Stun	3.24	0.080

4.3.3.3 Fillet Moisture and Lipid

The samples taken from the fillets at the end of the experiment were analysed for moisture and lipid content (table 4.3.18). A one factor ANOVA showed that there was no significant differences between groups in either moisture or lipid content ($p<0.05$). Thus both of these could be ruled out as factors causing differences between the groups. However, the level of lipid in the fillets was very high. Normal industry standards specify flesh lipid levels of between 10 and 14% (pers comm. Mark Osborne, Acorn Smokeries Plc., Chilcompton, U.K.), but the table shows that the mean lipid content was much higher in this trial. This was caused by the diet which the fish had been fed causing them to deposit large amounts of lipid in their flesh.

Table 4.3.18 Mean fillet moisture and lipid content after storage.

Slaughter Method	Moisture (%)	s.e.m.	Lipid (%)	s.e.m.
AQUI-S™	62.2	0.30	17.1	0.42
Carbon Dioxide	62.0	0.39	17.3	0.46
Percussion Stun	61.8	0.29	17.5	0.38
Crowd and Stun	61.4	0.28	17.9	0.39

4.3.3.4 Fillet Temperature

The fillet temperature was taken with the same precautions as for the first experiment. Table 4.3.19 shows the mean temperatures of the fish at each measurement point. A one factor ANOVA showed that there were no significant differences between the temperature of the fillets in each group at any point in time. This allowed temperature to be ruled out as having an effect on the results.

Table 4.3.19 The change in mean temperature of the fillets from the four slaughter groups.

Time (Hours)	Mean Temperature (± s.e.m.) for Each Slaughter Method (°C)			
	AQUI-S™	Carbon Dioxide	Percussion Stun	Crowd and Stun
5.7	3.7 (0.19)	4.5 (0.62)	3.5 (0.20)	3.5 (0.18)
11.5	-0.1 (0.09)	0.0 (0.06)	0.1 (0.11)	-0.1 (0.09)
20.2	-0.5 (0.07)	-0.3 (0.07)	-0.5 (0.06)	-0.5 (0.04)
24.5	-0.2 (0.04)	-0.1 (0.07)	-0.2 (0.05)	-0.2 (0.04)
36.0	-0.6 (0.06)	-0.6 (0.06)	-0.5 (0.07)	-0.7 (0.05)
48.5	0.3 (0.05)	0.3 (0.05)	0.4 (0.07)	0.3 (0.05)
54.5	-0.1 (0.05)	0.0 (0.04)	0.0 (0.06)	-0.1 (0.04)
72.0	0.0 (0.03)	-0.1 (0.06)	0.0 (0.04)	0.0 (0.02)
95.0	-0.1 (0.05)	-0.2 (0.02)	-0.2 (0.04)	-0.2 (0.02)

Figure 4.3.16 shows the change in temperature over time. The fillets cooled rapidly over the first eleven hours to just below 0°C and then remained at approximately the same temperature throughout the rest of the storage period.

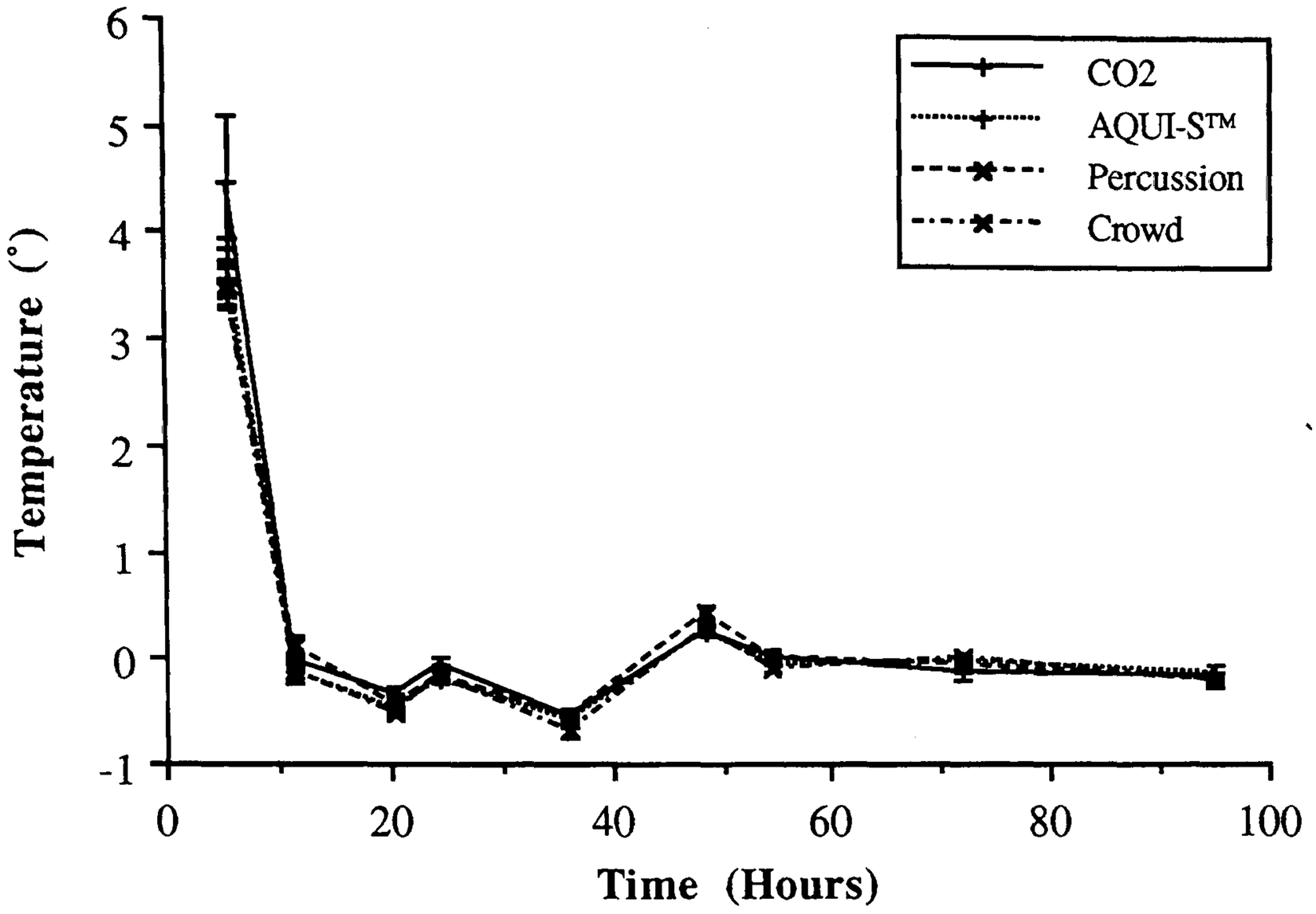

Figure 4.3.16 Changes in mean fillet temperature with storage.

4.3.3.5 Fillet pH

Care was taken with the measurements of the muscle pH as before. The fillets were thoroughly wiped with dry tissue paper before the measurements to remove any water and exudate that had collected between sample points. Table 4.3.20 shows the mean muscle pH of each group at each time point. The mean was calculated by converting the pHs to hydrogen ion concentrations as before. A single factor ANOVA was performed on the hydrogen ion concentration at each point in time and the results of this are shown in the table.

Table 4.3.20 Mean muscle pH values at each measurement point. Values in the same row with different superscript letters are significantly different ($p<0.05$).

Time (Hours)	Mean pH (± s.e.m.) for Each Slaughter Method			
	AQUI-S™	Carbon Dioxide	Percussion Stun	Crowd and Stun
5.7	6.83 (0.073) ^{a}	6.45 (0.041) ^{b}	7.16 (0.033) ^{c}	6.71 (0.069) ^{a}
11.5	6.77 (0.090) ^{a}	6.41 (0.027) ^{b}	7.00 (0.088) ^{a}	6.56 (0.065) ^{c}
20.2	6.56 (0.065) ^{ac}	6.33 (0.019) ^{b}	6.67 (0.061) ^{a}	6.45 (0.044) ^{c}
24.5	6.49 (0.047) ^{a}	6.34 (0.031) ^{b}	6.50 (0.038) ^{a}	6.34 (0.028) ^{b}
36.0	6.35 (0.015) ^{ab}	6.31 (0.015) ^{ac}	6.36 (0.010) ^{b}	6.30 (0.013) ^{c}
48.5	6.33 (0.011) ^{a}	6.36 (0.017) ^{a}	6.35 (0.016) ^{a}	6.33 (0.023) ^{a}
54.5	6.28 (0.014) ^{ab}	6.24 (0.015) ^{a}	6.28 (0.010) ^{b}	6.29 (0.016) ^{b}
72.0	6.32 (0.013) ^{ac}	6.28 (0.017) ^{b}	6.32 (0.007) ^{ac}	6.30 (0.011) ^{bc}
95.0	6.39 (0.014) ^{a}	6.37 (0.015) ^{a}	6.39 (0.013) ^{a}	6.36 (0.013) ^{a}

From table 4.3.20 it can be seen that initially there was a significant difference between the straight percussion stun group and the crowd and stun group. The percussion stun group also had a significantly higher pH than the AQUI-S™ anaesthetised group. All groups had significantly higher pH values than the carbon dioxide anaesthetised group immediately at the first sample point. This lasted until 24 hours after slaughter, when the crowded fish had reached the same low pH. By

48 hours after slaughter, all groups had the same muscle pH and remained at this level apart from small fluctuations until the end of the experiment.

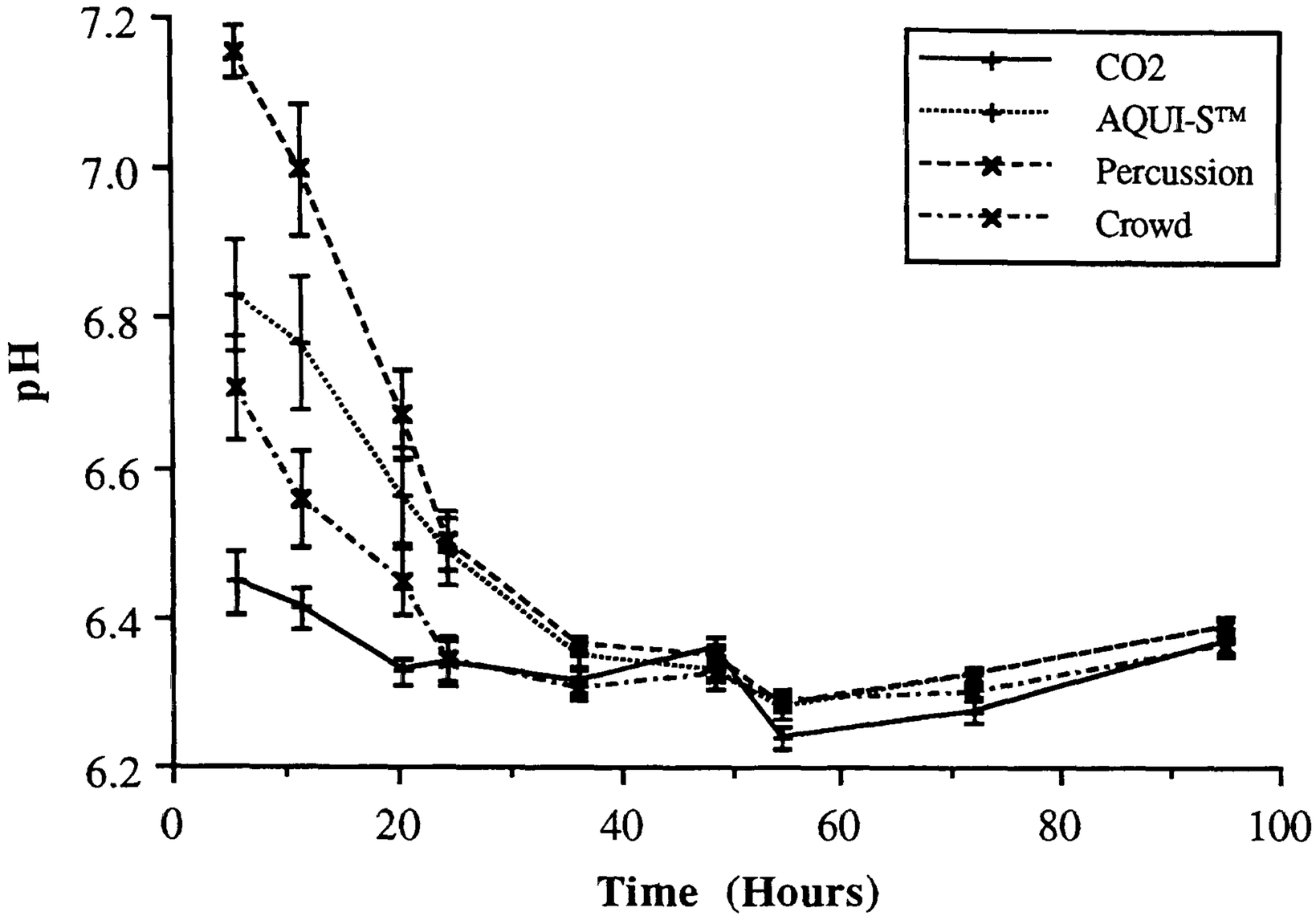

Figure 4.3.17 Changes in mean muscle pH (± s.e.m.) during storage post-slaughter

Figure 4.3.17 shows that the carbon dioxide anaesthetised fish had a very low initial pH and this only drops slightly before the terminal pH is reached. The pH of the percussion stunned group was much higher than that of the other groups at the start, but fell over the first twenty hours to reach the same level as the AQUI-S™ fish. The AQUI-S™ and the crowded fish started at similar levels, but the crowded fish showed a faster drop in pH reaching the terminal pH about ten hours earlier than the AQUI-S™ and percussion stunned fish.

The fall in pH of the AQUI-S™ fish was much faster than in the previous experiments in this study. It was also faster than the previous work carried out on salmon (pers. comm. Jan Holland, Crop and Food Research, Lower Hutt, New Zealand). It was expected that the pH would start to drop below 7.0 after about 24 hours, reaching the terminal pH after 50 to 60 hours.

4.3.3.6 Chroma Meter Measurements

The fillet lightness was described by the L* readings from the chroma meter. The mean L* values for each group and the results of a one factor ANOVA performed on the results at each point in time are shown in table 4.3.21.

Table 4.3.21 Mean fillet lightness at each sample point. Values in the same row with different superscript letters are significantly different ($p<0.05$).

Time (Hours)	Mean L* Value (± s.e.m.) for Each Slaughter Method			
	AQUI-S™	Carbon Dioxide	Percussion Stun	Crowd and Stun
5.7	44.57 (1.206) ^{a}	46.80 (0.799) ^{a}	45.65 (1.214) ^{a}	45.34 (0.998) ^{a}
20.2	46.73 (1.429) ^{a}	49.65 (1.068) ^{ab}	47.81 (1.116) ^{ab}	50.58 (0.920) ^{b}
24.5	47.40 (1.136) ^{a}	48.62 (1.324) ^{a}	47.54 (1.083) ^{a}	48.75 (0.967) ^{a}
36.0	49.89 (1.287) ^{a}	54.16 (1.163) ^{b}	50.26 (1.088) ^{a}	48.74 (1.138) ^{a}
48.5	49.31 (0.990) ^{a}	48.80 (1.313) ^{a}	48.10 (1.149) ^{a}	47.97 (0.864) ^{a}
54.5	50.36 (0.974) ^{a}	50.33 (1.331) ^{a}	49.23 (1.070) ^{a}	49.54 (1.155) ^{a}
72.0	49.39 (0.986) ^{a}	49.15 (0.894) ^{a}	47.45 (0.894) ^{a}	47.43 (0.808) ^{a}
95.0	48.95 (1.086) ^{a}	48.95 (0.864) ^{a}	49.42 (0.705) ^{a}	49.35 (0.733) ^{a}
168.0	43.43 (0.632) ^{a}	44.06 (0.338) ^{a}	44.76 (0.574) ^{a}	43.56 (0.444) ^{a}

There were very few differences in lightness between the groups (table 4.3.21). At two points one of the groups with higher activity showed significantly higher L* scores. However, the values were mainly unaffected by the slaughter treatment.

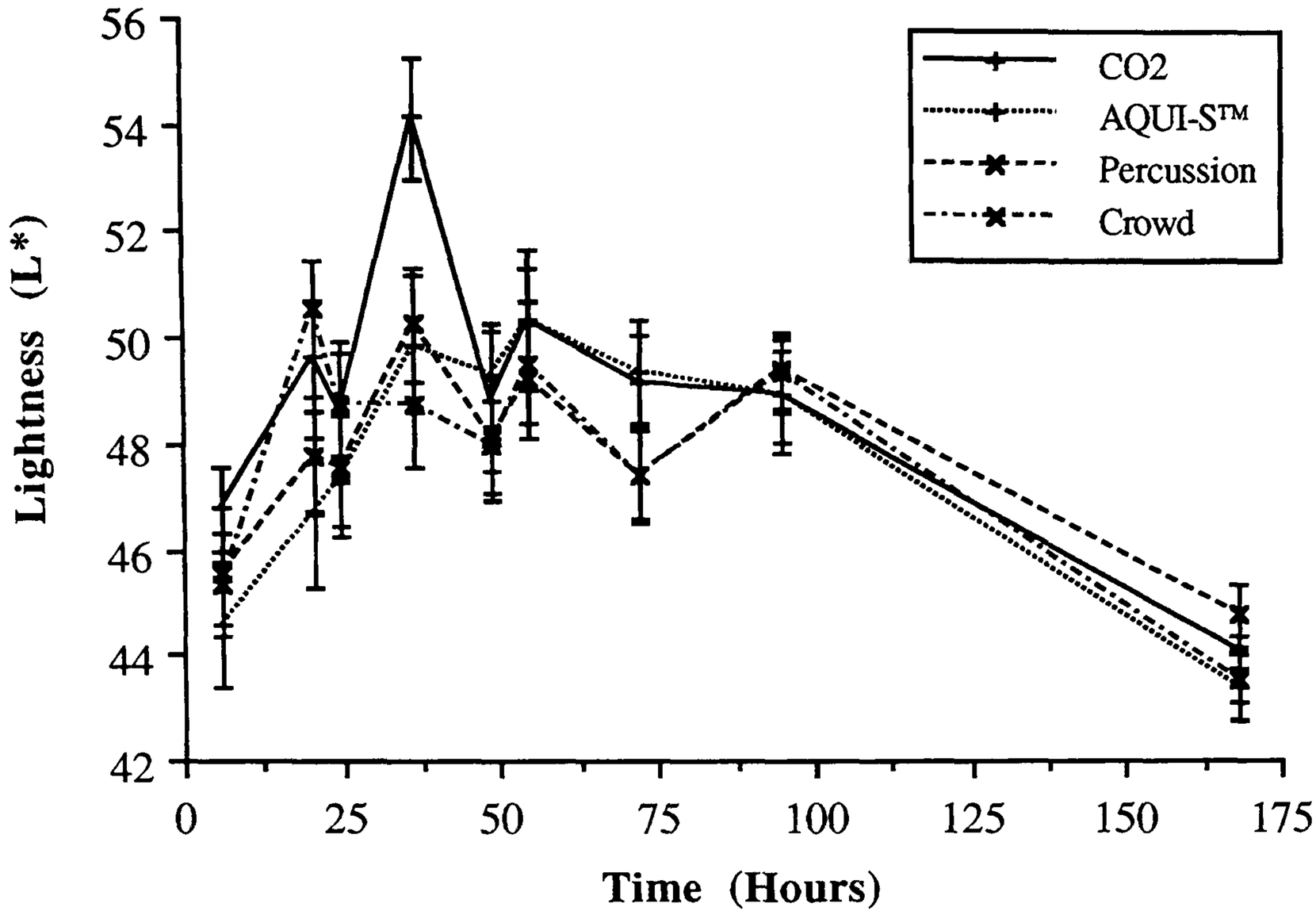

Figure 4.3.18 Changes in mean fillet lightness (± s.e.m.) with time after slaughter

The L* values of all groups rose by approximately six units over the first fifty hours of storage (figure 4.3.18). During the next fifty hours the values were approximately constant, before dropping again to approximately their original value. From the figure it is clear how little difference in flesh lightness there was between the groups.

The angle of hue was calculated from the a* and b* values as before. The mean values for the slaughter groups are shown in table 4.3.22. The results of a one factor ANOVA at each time point are also shown in the table.

Table 4.3.22 Mean fillet angle of hue throughout the trial. Values in the same row with different superscript letters are significantly different ($p<0.05$).

Time (Hours)	Mean Angle of Hue (± s.e.m.) for Each Slaughter Method (°)			
	AQUI-S™	Carbon Dioxide	Percussion Stun	Crowd and Stun
5.7	49.49 (0.918) ^{a}	50.43 (0.650) ^{a}	50.20 (0.453) ^{a}	48.46 (0.809) ^{a}
20.2	49.99 (0.708) ^{a}	52.25 (0.431) ^{b}	50.19 (0.720) ^{ac}	51.81 (0.521) ^{bc}
24.5	50.49 (0.731) ^{a}	50.75 (0.778) ^{a}	49.86 (0.749) ^{a}	50.73 (0.616) ^{a}
36.0	50.55 (0.852) ^{ab}	52.28 (0.486) ^{a}	51.86 (0.447) ^{ab}	50.20 (0.717) ^{b}
48.5	50.20 (0.366) ^{a}	49.27 (0.488) ^{a}	49.20 (0.431) ^{a}	48.95 (0.531) ^{a}
54.5	51.13 (0.422) ^{a}	50.65 (0.565) ^{a}	50.46 (0.422) ^{ab}	49.15 (0.560) ^{b}
72.0	49.26 (0.453) ^{a}	48.74 (0.470) ^{a}	48.22 (0.342) ^{a}	48.20 (0.436) ^{a}
95.0	48.30 (0.355) ^{a}	47.89 (0.429) ^{a}	48.03 (0.326) ^{a}	47.78 (0.301) ^{a}
168.0	48.98 (0.459) ^{a}	49.15 (0.349) ^{a}	48.53 (0.398) ^{a}	48.47 (0.338) ^{a}

There were very few significant differences between the angle of hue of the groups (table 4.3.22). A few individual values were significantly different, but no trends were shown, so these differences probably occurred by chance.

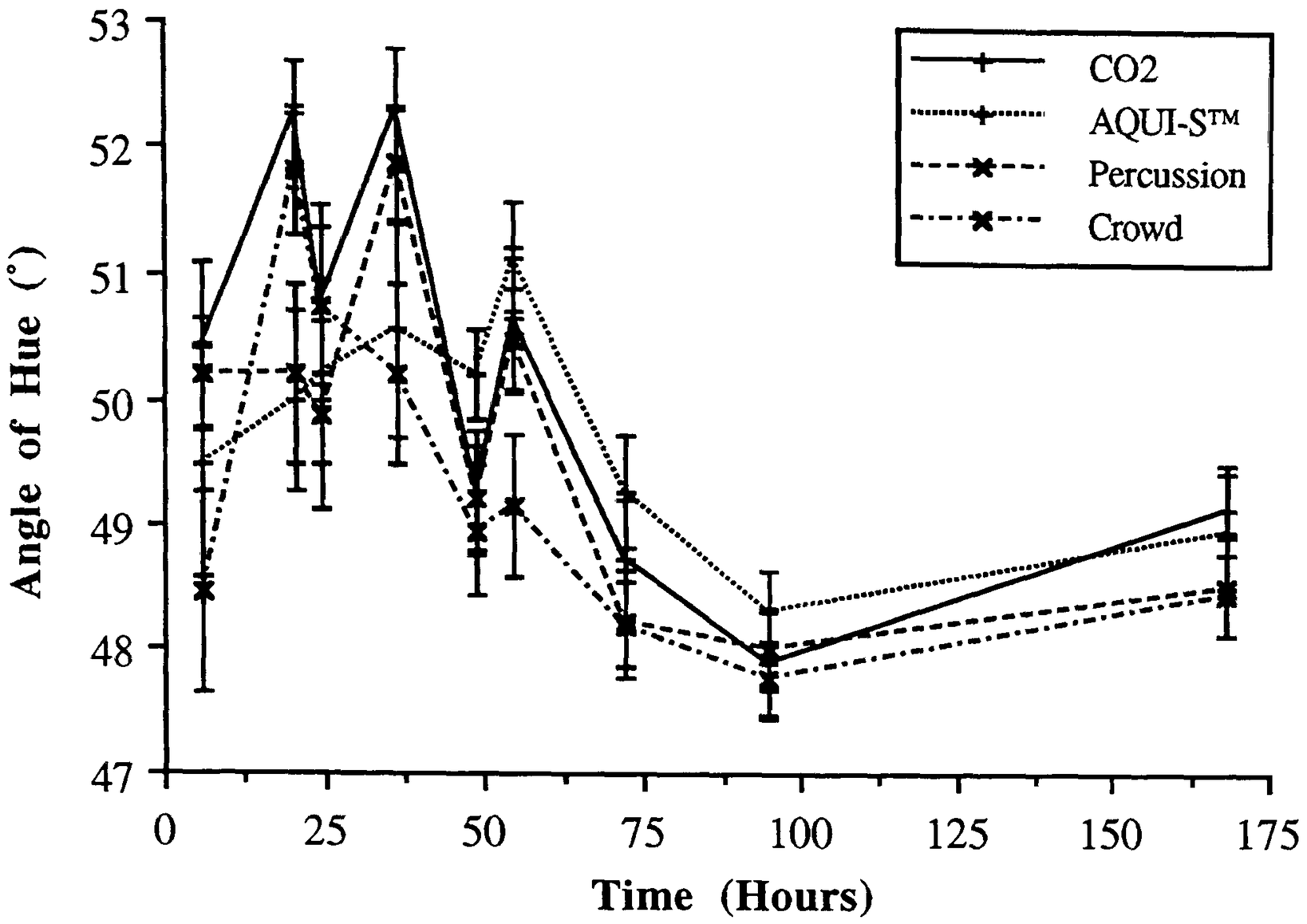

Figure 4.3.19 Changes in fillet angle of hue (± s.e.m.) with time after slaughter.

From figure 4.3.19 the similarity between the groups can be seen. Initially there was a lot of variation within groups, shown by the large standard errors, but this reduced with storage. During the first forty hours the angle of hue increased by a few degrees, indicating that the colour of the fillets became more yellow. The angle then dropped over the next fifty hours and stabilised at a more red colour for the remainder of the trial.

The fillet chroma was calculated from the a* and b* values as before. The mean values are shown in table 4.3.23 and the results of one factor ANOVAs at each sample point are also displayed.

Table 4.3.23 Mean fillet chroma values. Values in the same row with different superscript letters are significantly different ($p<0.05$).

Time (Hours)	Mean Angle of Hue (± s.e.m.) for Each Slaughter Method (°)			
	AQUI-S™	Carbon Dioxide	Percussion Stun	Crowd and Stun
5.7	18.31 (0.653) ^{a}	16.23 (0.764) ^{b}	17.62 (0.469) ^{ab}	16.76 (0.504) ^{ab}
20.2	17.96 (0.997) ^{a}	19.92 (1.045) ^{ab}	18.29 (0.797) ^{a}	20.96 (0.915) ^{b}
24.5	18.03 (0.795) ^{a}	18.93 (0.975) ^{a}	17.58 (0.752) ^{a}	19.72 (1.036) ^{a}
36.0	19.45 (1.103) ^{a}	21.56 (0.995) ^{a}	19.60 (0.752) ^{a}	19.94 (0.934) ^{a}
48.5	19.14 (0.774) ^{a}	18.37 (0.887) ^{a}	17.32 (0.738) ^{a}	18.36 (0.593) ^{a}
54.5	19.72 (0.969) ^{a}	18.78 (1.058) ^{a}	17.73 (0.710) ^{a}	18.46 (0.763) ^{a}
72.0	19.28 (0.694) ^{a}	19.22 (0.752) ^{a}	17.69 (0.636) ^{a}	18.73 (0.578) ^{a}
95.0	19.12 (0.638) ^{a}	19.11 (0.751) ^{a}	18.78 (0.485) ^{a}	19.39 (0.445) ^{a}
168.0	18.31 (0.383) ^{a}	18.27 (0.774) ^{a}	17.42 (0.622) ^{a}	18.69 (0.617) ^{a}

There were very few significant differences between the groups (table 4.3.23). At the initial sample point the carbon dioxide anaesthetised group had a significantly lower chroma than the AQUI-S™ anaesthetised group, but this difference disappeared by the next sample point, when the crowded group had a significantly higher chroma than the percussion stunned and the AQUI-S™ groups. From twenty hours after slaughter there were no further differences between the groups.

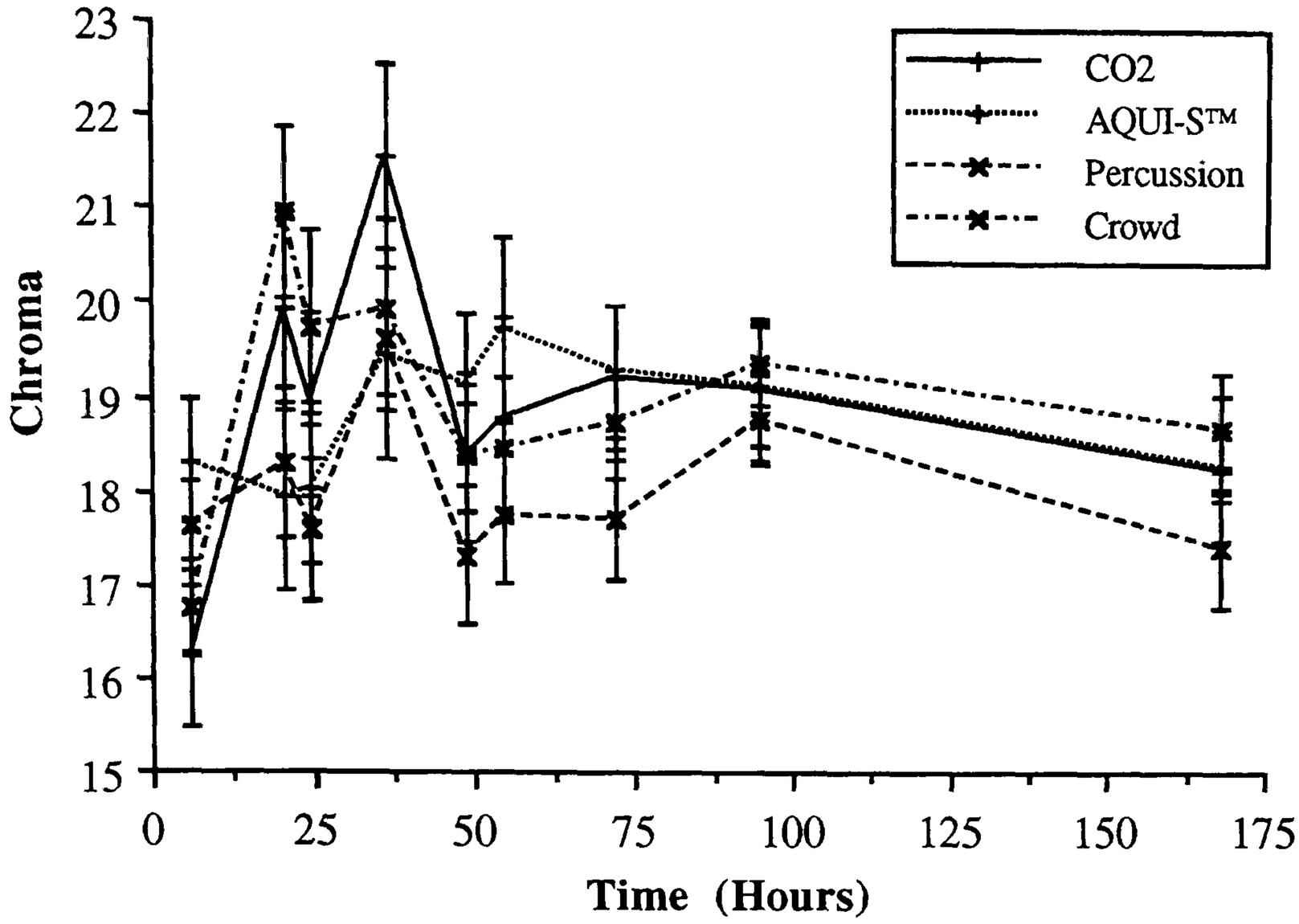

Figure 4.3.20 Changes in fillet chroma (± s.e.m.) with time after slaughter.

There was a great deal of variation within groups during the first fifty hours. The standard errors for each group are large up to that point and then decrease.

Figure 4.3.20 also shows that the chroma of the fillets rose slightly during the first fifty hours, and then stabilised for the rest of the trial. This indicated that the fillets became slightly more opaque during that initial period.

4.3.3.7 Roche Colour Card Scores

As the two measurements were carried out under different conditions and by different assessors, the two sets of results cannot be compared. The reason for the different conditions was that there was no light box in Bristol, where the fillets were stored after the initial measurements.

Table 4.3.24 shows the mean scores for each group at the two points in time. Also shown in the table are the results of a Mann-Whitney U-test between the groups.

Table 4.3.24 Mean Roche colour card scores. Values in the same column with different superscript letters are significantly different ($p<0.05$).

Slaughter Method	Mean Roche Colour Card Score (± s.e.m.)	
	6 Hours	168 Hours
AQUI-S™	13.6 (0.09) ^{ab}	14.7 (0.17) ^{a}
Carbon Dioxide	13.8 (0.13) ^{a}	15.0 (0.12) ^{ab}
Percussion Stun	13.6 (0.09) ^{ab}	15.1 (0.17) ^{ab}
Crowd and Stun	13.5 (0.10) ^{b}	15.2 (0.11) ^{b}

There was little difference in the colour card scores between the groups. A significant difference was observed initially between the carbon dioxide anaesthetised fish and the crowded fish, but this had disappeared by the end of the trial, when a difference between the AQUI-S™ and the crowded fish appeared. The causes of these differences is not obvious and may just be due to random chance.

The most obvious information gained from the table is the low scores given to the fillets. High quality fillets for commercial sale should have a Roche colour card score of at least 15 (Anon., 1995). The mean score was initially 13.5, which would have led to the rejection of that entire batch. The low scores were probably due to the very high fat levels in the fillets as discussed above.

4.3.3.8 Rigor

The mean angle of droop of the tail for each group is shown in table 4.3.25. The mean angles were compared using a one factor ANOVA at each time point. The results of these analyses are also shown on the table.

Table 4.3.25 Mean angle of droop for each group. Values in the same row with different superscript letters are significantly different ($p<0.05$).

Time (Hours)	Mean Droop (± s.e.m.) for Each Slaughter Method (°)			
	AQUI-S™	Carbon Dioxide	Percussion Stun	Crowd and Stun
3.7	49 (1.4) ^{a}	42 (2.4) ^{b}	47 (2.4) ^{ab}	47 (1.6) ^{ab}
11.5	30 (3.5) ^{a}	9 (3.5) ^{b}	22 (3.7) ^{a}	19 (4.1) ^{ab}
20.2	20 (4.3) ^{a}	7 (0.6) ^{b}	12 (1.8) ^{b}	9 (2.2) ^{b}
24.5	17 (4.0) ^{a}	12 (1.9) ^{a}	11 (2.2) ^{a}	11 (2.3) ^{a}
36.0	15 (1.9) ^{ab}	21 (2.4) ^{a}	10 (1.5) ^{b}	15 (3.0) ^{ab}
48.5	22 (2.5) ^{a}	35 (1.2) ^{b}	24 (1.9) ^{a}	30 (3.3) ^{ab}
54.5	35 (3.2) ^{a}	45 (1.3) ^{b}	35 (2.0) ^{a}	36 (2.5) ^{a}
72.0	37 (1.7) ^{a}	43 (1.5) ^{b}	36 (1.5) ^{a}	37 (1.5) ^{a}
95.0	47 (2.2) ^{a}	49 (1.9) ^{a}	49 (1.9) ^{a}	48 (2.5) ^{a}

The carbon dioxide anaesthetised fish went into rigor very rapidly (figure 4.3.21). If an angle of droop of less than 20° indicated rigor, then these fish entered rigor in less than 10 hours and the crowded fish entered just after. The percussion stunned fish went into rigor after approximately 15 hours and the AQUI-S™ anaesthetised fish after about 24 hours.

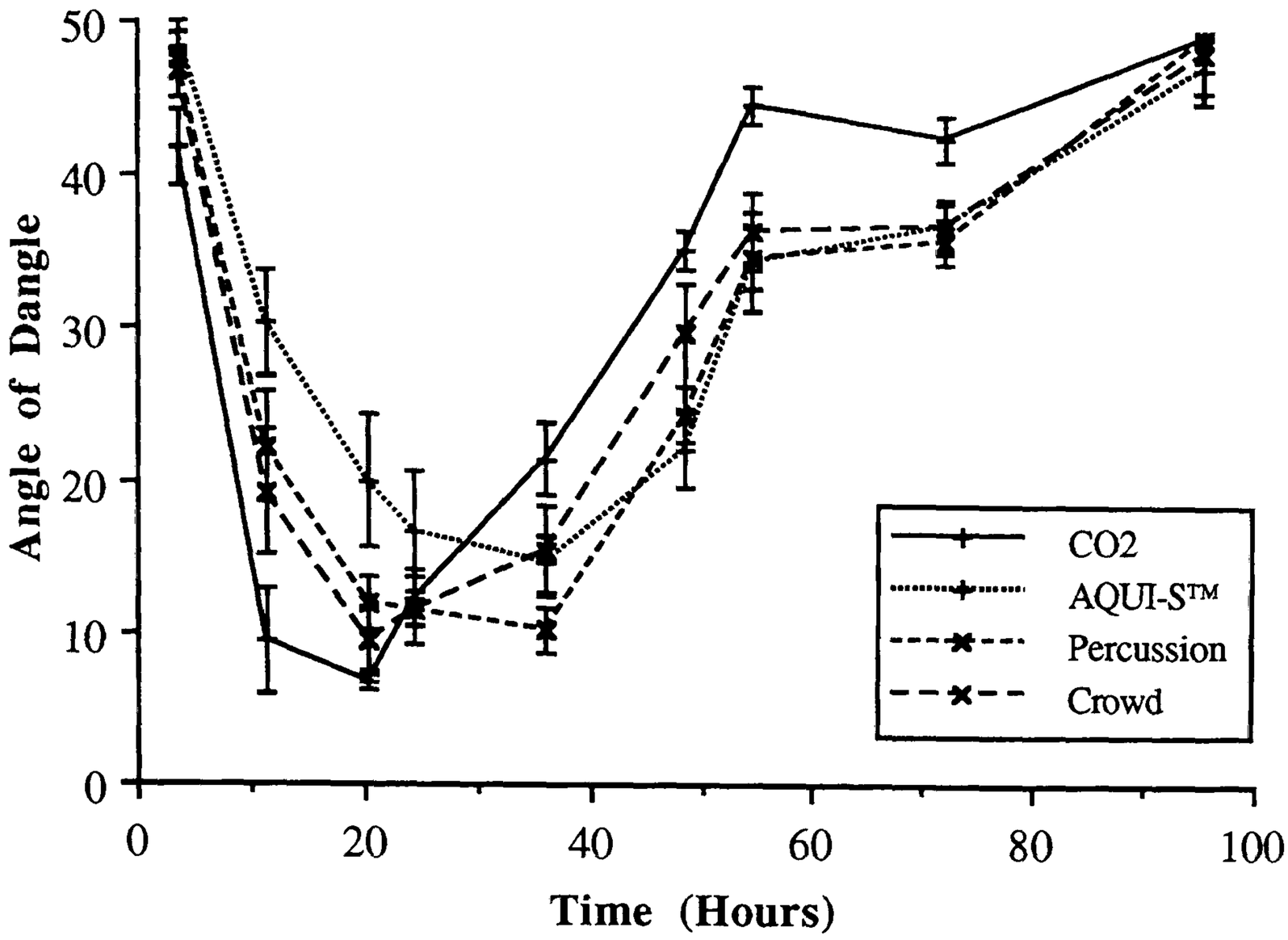

Figure 4.3.21 Changes in the state of rigor of the fish as observed by the angle of droop (± s.e.m.) of the tail of the fish.

The carbon dioxide anaesthetised fish came out of rigor first, at about 40 hours after slaughter, followed by the crowded group at 45 hours and the AQUI-S™ and percussion stunned fish at approximately 50 hours after slaughter. By 85 hours after slaughter all fish were able to droop over an angle greater than 40°, which had been the starting angle.

4.4 Discussion

The commercial use of AQUI-S™ during the pre-slaughter handling of salmon has been shown to result in slow falls in muscle pH post-mortem (pers comm. J. Holland, Food and Crop Research Ltd.). Typically, in commercial situations it should take between thirty and forty hours for the fish to reach a terminal muscle pH of approximately 6.4 in order to allow the fish to be processed before the onset of rigor. However, this may be reduced by stress to about twenty hours.

In the first experiment the anaesthetised fillets took 44 hours to reach the terminal pH of approximately 6.6. In the second experiment this took 30 hours and only 20 hours in the third, which was comparable with the netted group in the first experiment. The reasons for the faster fall in pH of the anaesthetised groups in the second and third experiments are not known. There were no apparent extra stressors imposed on the fish in the second experiment. In the third experiment the fish had been handled several times during the week before the experiment, but were left unhandled for the three days leading up to slaughter, which should have allowed sufficient time for recovery.

Previous work on stress at slaughter of fish has concentrated on the long term differences in muscle biochemistry and appearance. In such experiments measurements of pH were occasionally carried out after the pH had fallen to the terminal level (for example Azam *et al.* 1989 and Eifert *et al.*, 1992). From this, it was concluded that there was no effect of killing method on muscle pH as the terminal pHs quoted in these works were the same for the different methods of slaughter. Azam *et al.* (1989), measuring the pH before it reached its terminal value, found that percussion stunning resulted in a slightly higher pH than carbon dioxide anaesthesia, but the difference was very small, even though the measurements were taken six hours after slaughter.

Boyd *et al.* (1984) clearly showed the importance of making measurements of pH as soon as possible after slaughter. Thus they were able to show the different rate of fall of pH between kahawi (*Arripis trutta*) which had either been spiked or left to die in ice slurry. Fish in the spiked group were killed instantly and showed a slower fall in pH. This group also showed a longer time to the loss of ATP from the muscle — matched by a build up of IMP — even though both groups of fish started at the same initial concentrations of the two nucleotides. Because rigor sets in when ATP is lost, it can be concluded that the onset of rigor must have occurred later in the spiked group.

Lowe *et al.* (1993) also showed that less stressed or less active fish took longer to enter rigor. Studying the snapper (*Pagrus auratus*) it was found that rested fish went into rigor after 16 hours compared to fish which had been caught and held on a longline for 2 hours, which entered rigor after 2 hours.

The above studies are supported by the current work on salmon and trout. Combining observations of behaviour prior to slaughter with the pH and rigor measurements it is clear that increasing activity results in a faster fall in pH post-mortem and in a shorter time to the onset of rigor. This is caused by the increase in lactic acid interrupting the production of ATP, causing the muscle fibres to lock.

From the studies of rigor in the second and third experiments of this chapter, it can be seen that the anaesthetised fish do not achieve as low an angle of droop as the high activity fish when they are in full rigor. The softer feel of 'unstressed' fish in rigor compared to 'stressed' fish was also recorded by Erikson (1997) using a subjective scoring of rigor. However, muscle samples taken from these fish showed that the anaesthetised group still had ATP present when scored in full rigor, while there was none in the 'stressed' group (Erikson, 1997). This gives an explanation for the less firm fish during rigor.

Rigor occurs in an individual muscle cell when the ATP in that cell has been used up. Individual cells take different lengths of time to use up the ATP and so will enter rigor at different times. It would appear that in the 'stressed' or high activity groups the cells rapidly stop synthesising ATP and hence this molecule is used up at a similar rate in all cells. Thus the majority of the muscle cells are in rigor at the same time in the 'stressed' fish and the flesh feels very stiff.

In the anaesthetised, or 'unstressed', fish the synthesis of ATP continues for a longer time post-mortem and so the molecules are used up more slowly. The lengthened period results in some cells using up their supply of ATP more rapidly than others and there is a greater variation in the time that individual cells take to enter and to clear rigor within the same fish. Thus not all the muscle cells are in rigor at any one time, hence the less stiff fish.

The colour of the flesh of salmonids is often measured in experiments as it is easy to measure and is an important quality parameter. However, chroma meter readings are often misunderstood and misinterpreted. The most common misuse is that of a* as an indication of redness and b* of yellowness. This was demonstrated by Eifert *et al.* (1992) who drew some strange conclusions from their results on the colour of hybrid striped bass. Thus carbon dioxide anaesthetised fish have more green fillets than those in control groups, and fish chilled before slaughter have more blue fillets according to these researchers.

The colour of the fillets should be described by the angle of hue. When this is done, more meaningful descriptions of the colour of the flesh can be given. Comparing the means quoted in the tables of Eifert *et al.* (1992) and converting them to the angle of hue, it appears that the carbon dioxide stunned fish had yellower fillets whilst those of the chilled group were redder in hue — both of which are more natural colours for fish flesh than the greens and blues described by the authors.

From the first experiment in this chapter it is clear that a rapid fall in pH results in a less red coloured flesh than that resulting from a slow fall in pH and that the exact rate of fall is important. Thus for the salmon experiment, where the pH fall in the AQUI-S™ anaesthetised fish was faster than that in the trout, there were no differences in the colour of the fillets from the different slaughter groups throughout the trial.

This hypothesis was supported by the results of the Roche colour card analyses. Thus in the trout where the pH fall of the AQUI-S™ anaesthetised group was slow, a difference was observed between the high and low activity groups and no effect was seen in the salmon experiment, where the pH fall was faster.

The chroma and lightness are even more sensitive to the rate of fall of pH. An effect on chroma and lightness was only found in the first experiment where the pH fall of the anaesthetised group was the slowest. The fall in the second experiment was faster and it was faster still in the third, where no effects on chroma and lightness were observed.

In the first experiment, where a difference was found between the groups, it was obvious that a greater degree of activity leads to an increase in the lightness of the flesh. This was shown by the increased L* values of the active groups compared to the anaesthetised group. This difference held throughout the trial.

The effect on the flesh chroma was also clear from the first trial. The more active groups have a higher chroma. This shows that the flesh of fish which were active pre-slaughter are more opaque than that of anaesthetised fish.

The causes of the differences in colour, lightness and chroma are not clear from this series of experiments. Further work will have to be carried out to decide what the

exact causes are, but from work in red meat species it is possible to form a hypothesis about them.

In red meat species, a rapid fall in muscle pH post-slaughter causes changes in protein conformation (Warriss, 1996). The higher acidity of the muscle causes the protein bonds to alter resulting in the formation of insoluble protein from the soluble fraction. As the structures change so do the properties of the muscle. Light reflected from the surface of the muscle tends to be scattered more than before the changes occurred. The scattering results in interference and the reflected light thus appears whiter than if it had been reflected from muscle where the changes had not occurred. Further, the changes in the protein structure tend to mask the pigment within the flesh which give it the characteristic red colour. The pigment, which is myoglobin in red meat species, may be present at the same levels in muscle which has experienced a rapid fall in pH, but the colour will be altered by the changes in the muscle structure.

The opacity of the flesh is effected in the same way. Light tends to be absorbed more by the altered muscle structure as the protein formations are not as ordered as before. This results in the flesh appearing more opaque than in unaltered muscle.

The increase in the amount of insoluble protein results in an increased loss of exudate, or drip, from the flesh during post-slaughter storage. The exudate is a water-based solution of salts which is lost from the carcass after storage. With a decrease in the amount of soluble protein in the muscle there is less attraction to hold the water within the carcass and so the loss of exudate increases. The loss of exudate from the flesh results in textural changes. The flesh becomes softer as there is less support for the muscle structure from the liquid.

The changes in colour of the fish muscle observed in the first two experiments indicate that a process similar to that observed in red meat may be occurring in the

flesh of fish post-slaughter. Fish which are subject to high levels of activity or stress at slaughter show a rapid fall in pH and an increased flesh lightness, chroma and angle of hue.

In order to confirm whether the process causing the change in colour is the same as that seen in red meat species, a further experiment must be carried out. This will investigate the amount of insoluble protein present in muscle samples from fish killed after undergoing different levels of activity. Such an experiment would determine whether muscle proteins are affected by the rate of fall of the pH post-slaughter in fish.

From the present work, it is suspected that the muscle proteins are probably more sensitive to the fall in pH than those in red meat species. It has already been shown that the three experiments resulted in three different rates of pH fall in the anaesthetised groups. The anaesthetised group in the first experiment took 44 hours to reach the terminal pH, 30 hours in the second experiment and 24 hours in the third. In the first experiment there were significant differences between the anaesthetised group and the high activity groups in the angle of hue, the lightness and the chroma. In the second experiment the angle of hue differed significantly, but the lightness and chroma were not significantly affected by pre-slaughter activity. However, plotting the graph of the means showed that the mean lightness and chroma for the anaesthetised group was lower than that of the high activity group. In the third experiment, there were no effects on colour.

This change in results over the three experiments shows that the rate of fall of muscle pH in the low activity group had to be very slow to observe a significant effect with small numbers of fish. The effect may still have been present with a slightly faster rate of fall, but with small numbers of fish it was not possible to observe it owing to the degree of natural variation in pigment levels within the fish. Therefore, in any

further work it is of great importance that the anaesthetised group should achieve a slow rate of fall of pH, taking at least 30 hours and preferably 40 hours to fall to the terminal pH value. This will allow any colour difference between the anaesthetised group and the high activity groups to be observed. A difference in muscle protein solubility and exudate loss can also be looked for.

If changes in muscle protein conformation are responsible for the differences in observed colour, then they may also be responsible for the increased susceptibility of the fillets to gaping. It is known that gaping is caused by the failure of the fibres under strain (Jerrett *et al*, 1996). If the structure of the proteins was altered and cross-links between proteins disrupted then the strength of the fibres may be weakened.

Flesh texture is very important to the fish industry as visual appearance of the flesh is used to assess quality. It is therefore important that future experiments investigate this effect as well as the effects on colour.

Chapter 5

The Effects of Vitamins C and E on Pigment Concentration and Eating Quality During Storage of Fillets on Ice

5.1 Introduction

In the previous chapter the importance of the colour of the fillets was discussed and it was demonstrated that fillet colour was affected by the level of activity at slaughter. Following slaughter fish are stored for a period of time before consumption and during this time further spoilage of the flesh may occur, caused by enzymes, bacteria or oxidation (Quarmby and Ratkowsky, 1988; Connell, 1995). Anti-oxidants within the flesh tend to react with the free radicals produced by the oxidation process (reviewed by Undeland, 1995). As astaxanthin is a very strong anti-oxidant (Miki, 1991) it may be used up during the frozen storage of the fillets (work on rainbow trout by Bjerkeng and Johnsen, 1995; No and Storebakken, 1991; Chen *et al.*, 1984). However, Chen *et al.* (1984) found very little change in pigmentation during 14 days of storage at 1-2˚C in air in the dark, which seems surprising from the results of other authors and from their own results presented in the same paper for frozen fish and fillets stored under oxygen evacuated and CO_2 atmosphere packaging.

The increase of the products of oxidation in fish during storage is well documented (for example Bjerkeng and Johnsen, 1995). In red meat species, the flesh shows reduced effects of oxidation after feeding dietary supplements of anti-oxidants such as α-tocopherol (Liu *et al.*, 1995; Wood and Enser, 1997) or through the addition of anti-oxidants such as ascorbic acid and α-tocopherol to the flesh post-slaughter (Mitsumoto *et al.*, 1991). The anti-oxidants allow the meat to keep its bright red colour, caused by oxymyoglobin, which consumers associate with freshness by preventing the oxidation of oxymyoglobin to metmyoglobin which is brown in colour (Manutawaik *et al.*, 1991). Both dietary and post-slaughter additions have also been shown to have an effect on the oxidation of poultry flesh during storage (Sante and Lacout, 1994). Post-slaughter treatments have been used successfully for finfish to preserve a red skin colour in rock fish (*Sebastolobus alascanas*) (Wasson *et al.*, 1991).

The effects of storage on eating quality of the flesh of fish can also be marked. While the flavour of fresh fish may be "so bland that words fail the human assessor" (Quarmby and Ratkowsky, 1988), after spoiling during storage a range of strong and undesirable flavours are detected. These may occur from either oxidation, resulting in flavours such as *rancid*, *oily*, and *cardboard*, or from the formation of hypoxanthine and bitter peptides, giving the flavours *astringent*, *acid*, *acrid*, *burnt*, *bitter* and *sour* and have a strong effect on the overall perception of the product quality (Quarmby and Ratkowsky, 1988).

Several authors observed a synergistic effect of ascorbic acid and α-tocopherol reducing oxidation within the flesh when both compounds were added post-slaughter (Mitsumoto *et al.*, 1991; Wasson *et al.*, 1991). Astaxanthin's anti-oxidant capabilities may also reduce the rate of oxidation of lipids, with the pigment being used up in the process and becoming colourless (Weber and Grosch, 1976). The synergistic effect observed with the combination of ascorbic acid and α-tocopherol may lead to the protection of astaxanthin from oxidation. This could reduce losses of astaxanthin during storage of the fish on ice and also help to reduce the onset of off-flavours associated with oxidation. Ascorbic acid may also affect the texture during eating as it is involved in collagen biosynthesis which may result in a firmer texture.

This chapter aims to investigate the concentration of ascorbic acid and α-tocopherol in the flesh and their relationships with astaxanthin levels and the eating quality of cooked fillets of Atlantic salmon both before and after storage on ice. Different concentrations of the vitamins in the flesh will be achieved by feeding the fish different levels of ascorbic acid and α-tocopherol supplements in their diet for a period before slaughter. The vitamin and astaxanthin levels and the eating quality of the flesh will then be monitored for a given period of storage.

5.2 Method

5.2.1 Diets

With the aim of obtaining a wide range in the levels of vitamin C and E in the flesh at slaughter, five diets with different levels of the two vitamins were manufactured. The levels chosen for the control diet were based on the commercial specifications for salmonid diets, which are approximately 200ppm for vitamin C and 350ppm for vitamin E. The experimental diets had either much lower or much higher levels of the vitamins.

Vitamin E was added to the vitamin and mineral package in the diets as α -tocopherol and vitamin C as ascorbic acid polyphosphate (Rovimix Stay- C25%, F. Hoffmann-La Roche). Each vitamin and mineral package also had sufficient astaxanthin, in the form of Carophyll Pink® (F. Hoffmann-La Roche), added to obtain a post-manufacture level of about 75ppm (during the extrusion process some of the pigment is lost, so slightly more was added to the pre-mix). All of the vitamin and mineral packages were prepared by Dr Paul Beardsworth (Roche Products Ltd., Heanor, U.K.). In all other respects the diets were identical. They were manufactured from the same basic ingredients as the diets in chapter 3 (table 3.2.1). The diets were made by BOCM Pauls (Renfrew, U.K.) under the supervision of Richard McKinney.

After processing, the diets were analysed for oil, moisture, protein, astaxanthin, ascorbic acid and α-tocopherol content. The results of these analyses, which were carried out by Roche UK, Welwyn Garden City, are shown in tables 5.2.1 and 5.2.2.

Table 5.2.1: Vitamin and astaxanthin contents of the five diets after processing.

Cage	Diet	Astaxanthin (ppm)	Ascorbic Acid (ppm)	α-Tocopherol (ppm)
1	Control	76.3	202.7	336
2	Low C Low E	67.3	29.1	74.9
3	High C Low E	70.2	542.5	125.7
4	Low C High E	71.4	42.1	1170
5	High C High E	84.3	639.3	1138

Table 5.2.2: Proximate analysis of the five diets after processing.

Cage	Diet	Dry Matter (%)	Crude Protein (%)	Oil (%)	Ash (%)
1	Control	94.1	42.2	27.9	8.33
2	Low C Low E	96.7	44.5	26.6	8.98
3	High C Low E	98.3	42.9	27.7	8.81
4	Low C High E	94.5	42.6	28.9	8.87
5	High C High E	95.6	44.6	25.3	8.60

After processing, the diets were stored in a cool room until required at the farm site. One month's supply of food at a time was shipped to the site, allowing the diets to be kept in conditions which aided preservation of the vitamins and minimised oxidation of the oils.

5.2.2 Fish

A large population of Atlantic salmon had been grown on Marine Harvest McConnell's farm on Loch Eil, Scotland. The experimental fish were obtained from this population. They had been previously fed a diet pigmented with astaxanthin and canthaxanthin to a total amount of 80ppm.

When the fish were of a mean weight of approximately 2.3kg, six hundred of them were netted out at random, individually weighed and measured whilst anaesthetised

with benzocaine solution, and divided equally between five cages on the Feed Trials Unit (FTU) at Loch Eil. The cages on the FTU measured 5m x 5m x 5m, which gave a stocking density of about 2.5kgm^{-3}. This was much lower than commercial densities which are normally about 20kgm^{-3}. The cages were made from net mesh, suspended from a floating platform. This allowed the fish to be crowded by raising the nets. The fish could then be handled from the platform.

After allowing the fish to recover from the stress of the handling and transfer, they were fed a ration according to their weight and the water temperature. The ration was determined according to tables used by Marine Harvest McConnell for the feeding of their own commercial stock with diets of the same protein and oil content and was approximately 1% of the total weight of fish in the cage per day.

At the end of each week the ration was recalculated to allow for expected growth. Every four weeks the fish were starved for two days and then individually weighed and measured whilst under anaesthesia. This allowed their growth to be monitored and the feed rates to be recalculated to accurately match the requirements of the fish.

The growth monitoring was important as the fish on the low vitamin C diets risked becoming vitamin C deficient, which would have led to problems with their immune systems. This would have shown in poor growth rates and would have led to the termination of the feeding period at that point, before the condition of the fish deteriorated too greatly.

During the trial the fish in each cage were treated routinely against sea lice using Aquaguard. This proved ineffectual and later hydrogen peroxide was used to much greater effect on the lice, but this was much more stressful for the fish. All cages were treated identically at the same time.

After nine weeks of feeding the diets the sea lice infection was very heavy and mortalities were high in all of the cages. The fish were starved for seven days — a standard pre-harvest procedure to clear food from their gut, to minimise the chance of flesh contamination by the gut contents on evisceration. The fish were then slaughtered — two weeks earlier than had been planned.

At slaughter the fish in each cage were crowded by lifting the nets and thirty fish from each cage netted out by hand. The fish were killed by percussion stunning with a polypropylene priest followed by bleeding as described in chapter 3.

After thirty fish from each cage had been placed in one bin, a second group of thirty were taken from each cage, killed and tagged as before and placed in a second bin. The fish in the first bin were to be kept as fresh fish and those in the second were destined for smoking.

The bins of fish were taken to a nearby processing unit (Marine Harvest McConnell, Blar Mhor, Fort William). The slush ice was drained from the bins and the fish lifted out. Each fish was individually weighed and measured, eviscerated, washed and reweighed. The fish were then packed in ice to allow rigor to set in and resolve.

5.2.3 Fish Storage

After rigor had resolved, the fish were filleted. The two fillets from each fish were tagged to identify them to the original fish. A sample of flesh weighing approximately 80g was taken from the right fillet of each fish from the position shown in figure 5.2.1. The right fillets were then individually vac-packed and blast frozen and stored at -40˚C.

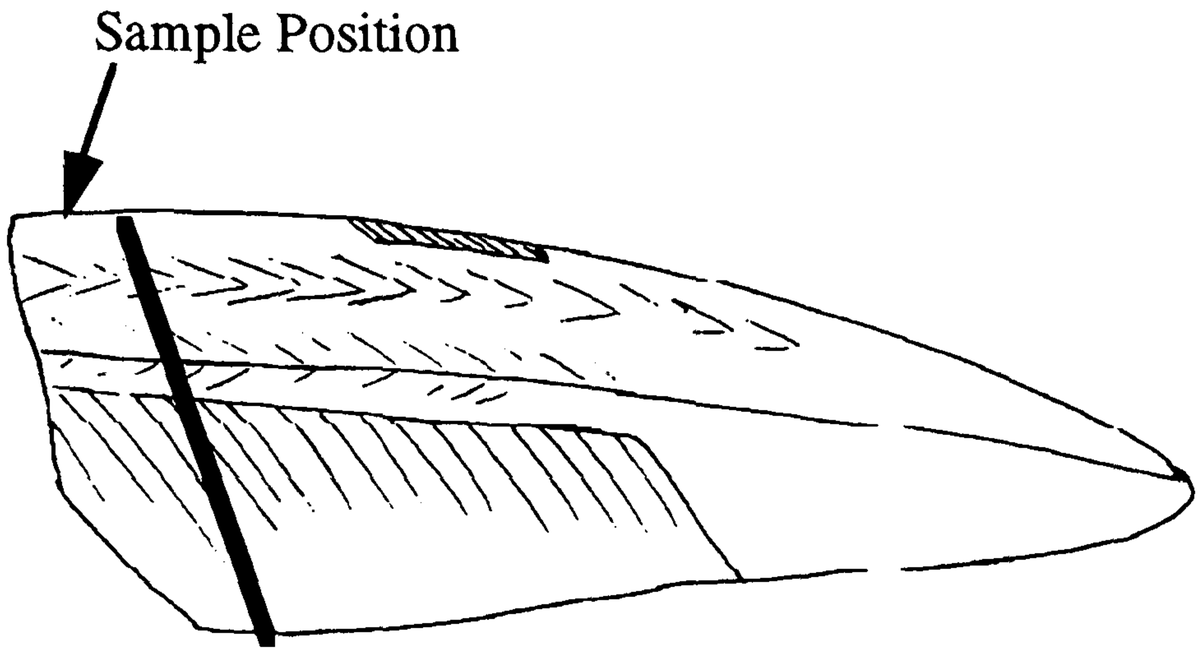

Figure 5.2.1 Position of cut to obtain samples.

The left fillets of each fish were placed on melting ice, separated from the ice and water by a layer of plastic, so that they were exposed to the air. The ice was in polystyrene fish boxes which were placed in a dark, chilled room kept at 4°C. The fish were stored under these conditions for 12 days. After 6 days and after 12 days of storage samples of flesh weighing approximately 50g were cut from the same region of the fillets as shown in figure 5.2.2. After 12 days of storage the fillets were individually vac-packed, blast frozen and stored at -40°C.

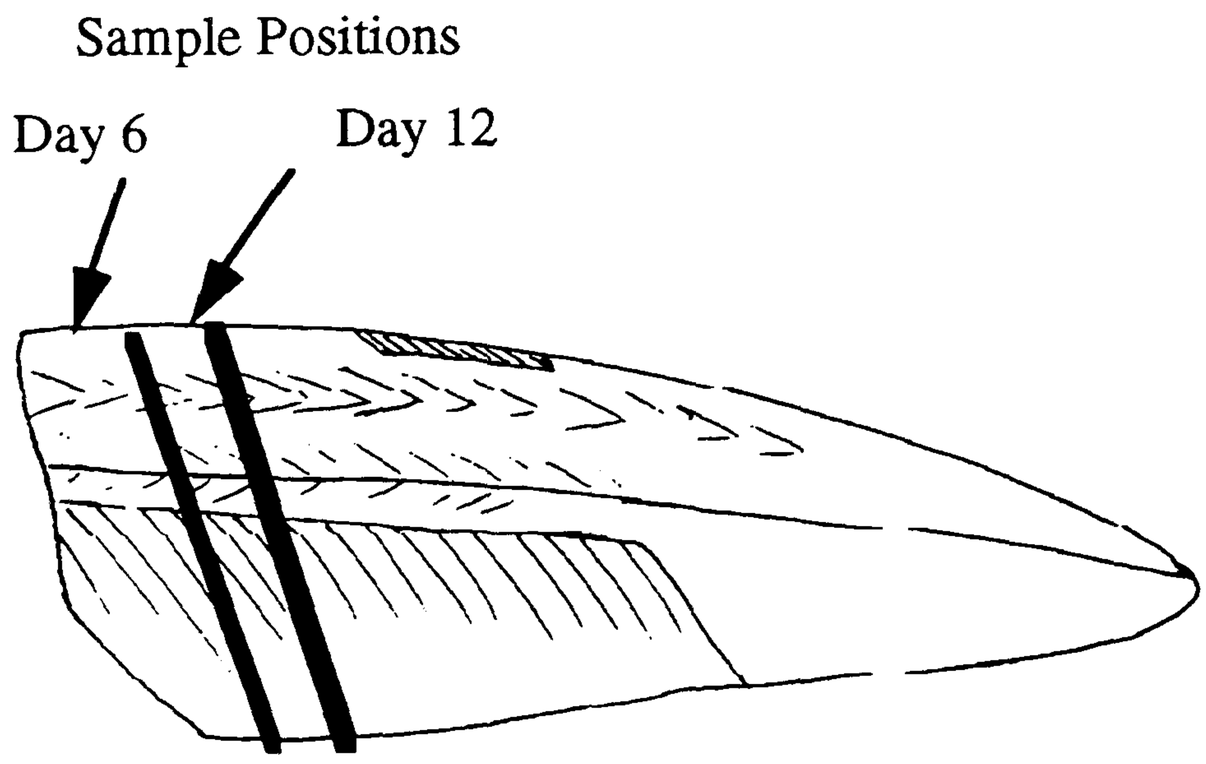

Figure 5.2.2 Position of samples taken after 6 and 12 days of storage.

5.2.4 Samples

All of the flesh samples were trimmed to remove the skin, red muscle and any parts of the dorsal fat depots and belly flaps. This left just the white muscle (figure 5.2.3). The first sample of white muscle taken from the right fillets (the 80g sample) was divided into four portions. Approximately 30g was bagged and frozen for fat content analysis. A sample of approximately 1g was accurately weighed into a 25ml universal container for vitamin C analysis. Two samples of approximately 20g were separately bagged and frozen for astaxanthin and vitamin E analyses. All samples were frozen at -40°C.

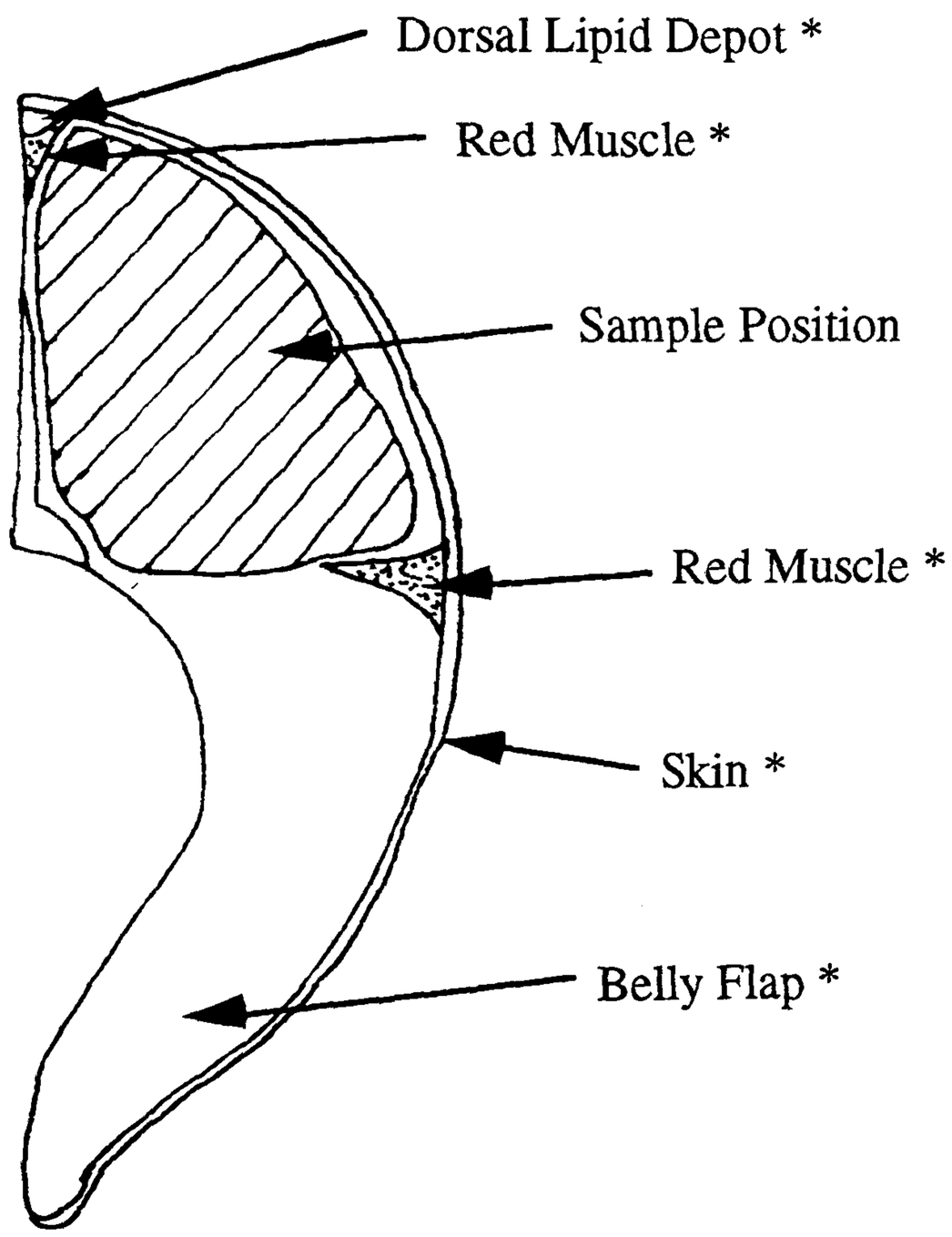

Figure 5.2.3 Position of sample after removal of tissues marked *.

The rest of the samples taken after 6 and 12 days of storage were divided into three — for vitamin C, vitamin E and astaxanthin analyses. These were then frozen and stored as above. The procedures for the analyses are described below.

5.2.5 Lipid Content Analysis

The 30g samples of flesh were thawed and blended using a Moulinette blender (Moulinex, France), until a smooth paste was achieved. Two 5g portions of this paste were then analysed for total lipid content using a CEM AVC80 moisture and lipid analyser (CEM Corporation, USA). The microwave oven was run on 95% power for three minutes and the weight loss of the sample used to determine the moisture content. The lipid was then extracted from the dried sample with dichloromethane (RH1010, Rathburns, UK) and returned to the microwave oven. This was run at full power for two minutes and the weight of the resultant used to calculate the lipid content of the sample.

5.2.6 Astaxanthin Analysis

The extraction method used for astaxanthin was adapted from Roche methodologies. The 20g sample of white muscle was blended using the Moulinette blender and 10g of the paste accurately weighed into a 100ml beaker. To this 5g hydrated magnesium sulphate (BDH, UK) was added, as a grinding agent, and 40ml acetone (RH1021, Rathburns, UK). The mixture was blended using a Polytron blender (Kinematica, Switzerland) at 20,000rpm for about 60 seconds. The mixture was then filtered under vacuum through a sintered glass filter into a 250ml round bottomed flask. The powder was returned to the beaker and extracted twice more with fresh acetone.

The acetone was evaporated off under vacuum over a water bath at 60°C. Before all the acetone was evaporated 20ml absolute ethanol (RH1022, Rathburns, UK) was added to the flask and evaporated off. If water still remained in the flask, more ethanol was added and evaporated off until no water remained after evaporation. The oil extract containing the astaxanthin was transferred from the flask to a 25ml volumetric flask, using a mixture of 14% acetone and 86% by volume 97% n-hexane

(RH1033, Rathburns, UK). The volume was made up to the mark with this mixture and subsamples of the solution filtered into 2ml HPLC vials. These were frozen at -40˚C until required.

Standards of astaxanthin were prepared from a stock solution. This was made from 10mg of astaxanthin (F. Hoffmann-La Roche, Basel, Switzerland) dissolved in 100ml of 9:1 hexane: chloroform (RH1009, Rathburns, UK). From this standard solutions of approximately 10ppm were made and filtered into 2ml HPLC vials. The response curve of the astaxanthin — the plot of astaxanthin concentration against the absorption — is linear and so the concentration of the astaxanthin in the samples could be determined directly from the standards.

The samples were run through a 15cm column packed with LiChrosorb, 5µm (159002A, BDH, UK) which had been pretreated with 1% phosphoric acid (BDH, UK) for 2 hours and then standards injected until the responses were repeatable. The mobile phase used was the mixture of 14% acetone and 86% n-hexane, run at a flow rate of 1.2ml / min using a Gilson isocratic system pump (Gilson, USA). The injection was performed by a Kontron 360 autosampler unit (Kontron Instruments Ltd., UK). Detection was carried out at 469nm by a Kontron 332 absorbance detector (Kontron Intstruments Ltd., UK), which resulted in a clear peak corresponding to the astaxanthin appearing at about 5 minutes after injection. The total run time for each sample was 8 minutes.

For all the chromatography techniques each sample vial was injected twice. Two vials had been prepared from each sample so a total of four chromatograms were obtained from each sample. The area under the peak was determined using a PC Integration Pack, version 3.94 (Kontron Instruments, UK) and converted to the concentration of the substance using the data obtained from the standards. The mean

of the four readings was determined and the concentration in the sample determined using the following formula:

Sample conc. = Mean reading * dilution factor / sample weight

5.2.7 Vitamin C Analysis

The vitamin C content of the 1g sample was determined according to the methods of Schüep and Keck (1990). The sample of white muscle was accurately weighed into a 25ml universal container and 5ml of metaphosphoric acid solution was added. The metaphosphoric acid solution was prepared by dissolving 5g washed metaphosphoric crystals (291904A, BDH, UK) in 1000ml distilled water to which 2g dithioerythritol (Sigma 8255, Poole, UK) had been added.

The sample was homogenised in the extractant and then transferred to a 25ml centrifuge tube. The sample was centrifuged for 10 minutes at 15,000rpm, with the temperature at -10°C. The supernatant was filtered through a 0.2µm PVDF filter (Whatman INC., USA) into 2ml sample vials and frozen at -40°C until ready for analysis by high performance liquid chromatography (HPLC).

Standards of ascorbic acid were prepared as follows. Approximately 2.5g l-ascorbic acid (Sigma, UK) was accurately weighed and dissolved in 1000ml metaphosphoric acid to achieve a known concentration of approximately 2500ppm. From this stock solution a range of concentrations were made. These were run on the HPLC and compared with some samples to determine roughly what the concentration of ascorbic acid was in each sample. Samples of a similar concentration were then used in the full runs. This had to be done as the response curve of ascorbic acid against absorption on the HPLC is not linear (Schüep and Keck 1990).

The chromatography was carried out using a 250mm x 4.6mm column packed with Techsphere ODS, 5µm (HPLC Technology, UK). The mobile phase used was 40ml hexane, 15ml acetate buffer and 1.5ml 1,5- dimethylhexylamine (D16, 129-2, Aldrich, UK), made up to 1000ml with distilled water. The acetate buffer consisted of 36.8g sodium acetate $3H_2O$ (102354X, BDH, UK) dissolved in 800ml distilled water with 101ml acetic acid (100016X, BDH, UK). The pH of the buffer was adjusted to 3.8 using the acetic acid and the volume made up to 1000ml with distilled water.

The sample and mobile phase were pumped into the column using the same pump as for astaxanthin and the injection was performed by the Kontron 360 autosampler unit. Detection was carried out at 254nm by the Kontron 332 absorbance detector over the run period of 35 minutes.

5.2.8 Vitamin E Analysis

Vitamin E was detected as α-tocopherol using a method adapted from that used by Roche Products Ltd., UK. In a darkened room the 20g sample was blended using a Moulinette blender, until a smooth paste was achieved. Approximately 15g of this paste was accurately weighed into a 500ml amber coated round bottomed flask. To this 80ml ethanol and approximately 2g of ascorbic acid (440065P, BDH, UK) were added to act as an anti-oxidant. The sample was then saponified using 20ml 50% aqueous potassium hydroxide (P/5645/17, Fisher Scientific, UK) under reflux for 30 minutes, or until all the flesh had been saponified. The flask was then removed and cooled to room temperature on ice.

The saponified material was transferred to a 500ml amber separator and the flask washed with 100ml distilled water and 20ml ethanol. The washings were added to the separator. The flask was then washed using 100ml n-hexane and the washings added to the separator which was shaken vigorously for 30 seconds. After the aqueous and

organic phases had separated, the aqueous phase was run off and the organic phase stored in a clean 500ml amber round bottomed flask. The aqueous phase was returned to the separator and twice more extracted with n-hexane. The organic phase extracts were bulked together and the aqueous phase discarded.

The organic phase was placed in the separator and washed with 100ml portions of water until all traces of alkali had disappeared. The aqueous washings were discarded and any remaining water removed by running the organic extract through a column of anhydrous sodium sulphate (S/6640/60, Fisher Scientific, UK) into a 250ml amber round bottomed flask. This was evaporated to dryness over a water bath at 60°C. The residue was redissolved in filtered hexane and transferred to a 10ml volumetric flask. The volume was made up to the mark and subsamples of this filtered through 0.2µm pore PVDF filters into 2ml amber HPLC vials. These were frozen at -40°C until ready to run on the HPLC column.

The same autosampler and pump units were used as for the astaxanthin assay. The flow rate was 1.6ml / min, giving a run time of 11 minutes past an ABI 980 fluorescence detector (Applied Biosystems, USA) detecting at 296nm.

From the result of the analyses the effect of initial levels of vitamin C and vitamin E on the levels of astaxanthin throughout the storage period could be found. The data were also used to determine the effect of the vitamins on eating quality of fresh flesh during storage.

5.2.9 Eating Quality

The results of chapter 2 had shown that lipid content had little effect on eating quality. Therefore when twenty fish from each diet were selected for taste panelling the panels were set up with random lipid contents. The fish were then cooked and panelled as in chapter 2.

The results of the taste panel were analysed to find the effect of the vitamin C and E concentrations on flavour in cooked fish, both fresh and after storage on ice.

5.3 Results

5.3.1 Live Weight

During the trial the fish suffered from an infestation of sea lice. This caused the fish to reduce their feed intake and ultimately to stop feeding completely. Further effects on feed intake were caused by the treatment of the lice with Aquaguard and hydrogen peroxide. The fish were not fed on the day prior to the treatment and they took several days to recover from the stress of the treatments.

The effects of the infestation and its treatments are shown in the growth curves for each dietary treatment (figure 5.3.1). The growth of the fish was rapid over the first four weeks. The infestation of lice occurred in about the sixth week of the trial and its effects were noticeable in the reduction in growth observed at the weighing of the fish in the eighth week. By the slaughter date all groups of fish had lost weight.

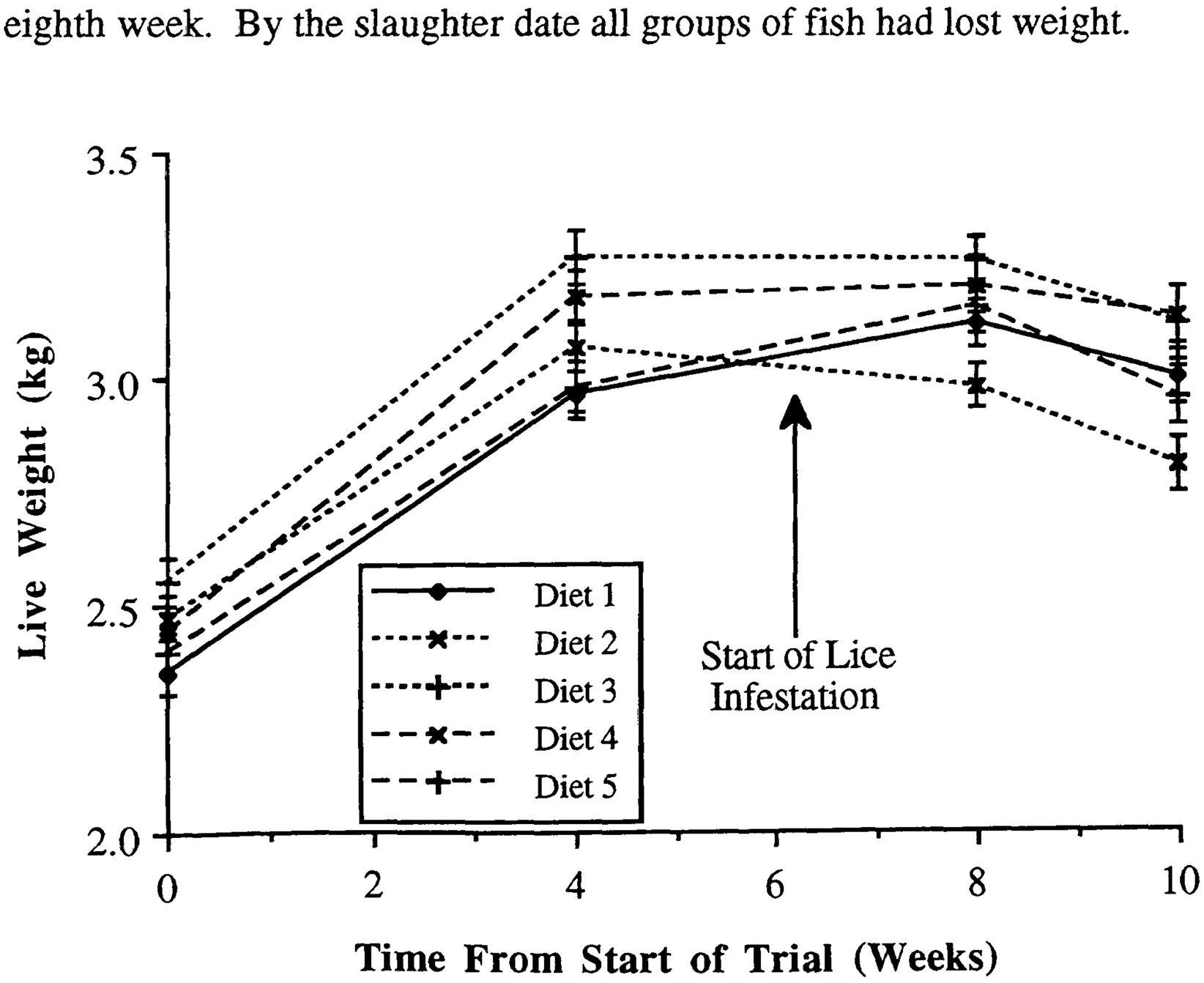

Figure 5.3.1 Growth of the fish (± s.e.m.) during the trial.

A one factor ANOVA (StatView) was performed at each weighing to observe whether there were any differences in mean weight between the cages (table 5.3.1). Significant differences in size occurred between the fish from the different cages, especially at slaughter. The fish in cage 2 were smaller than those in all other cages except for cage 5 ($p<0.05$). This could not be explained by just the sea lice infestation as cage 3 suffered the greatest mortalities during the trial (46 deaths between week 8 and slaughter in week 10), but had one of the highest mean live weights. This could have been caused by the sea lice resulting in the deaths of the smaller fish in cage 3, pushing the mean weight of that cage up. However, as the mortalities were not kept and weighed this will have to remain a speculation.

Table 5.3.1 Mean live weight (± s.e.m.) of the fish in each cage at each weighing point. Means in the same row with different superscript letters are significantly different ($p<0.05$).

	Live Weight (kg)				
Time (Weeks)	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5
0	2.35 (0.042)^{a}	2.47 (0.051)^{ab}	2.55 (0.054)^{b}	2.44 (0.048)^{ab}	2.40 (0.047)^{a}
4	2.96 (0.055)^{a}	3.07 (0.056)^{ac}	3.27 (0.061)^{b}	3.18 (0.060)^{bc}	2.98 (0.055)^{a}
8	3.12 (0.053)^{ab}	2.98 (0.049)^{a}	3.26 (0.055)^{b}	3.20 (0.062)^{b}	3.15 (0.057)^{b}
10	3.00 (0.059)^{bc}	2.81 (0.059)^{a}	3.12 (0.082)^{bc}	3.14 (0.063)^{c}	2.96 (0.065)^{ab}

The drop in weight between week 8 and week 10 occurred in all cages. This shows that no one particular diet resulted in a greater drop in weight. This had been a concern for diet 2 which had low levels of vitamin C and vitamin E.

The first thirty fish killed from each cage were placed into the same bin of ice slurry after slaughter. These fish were used for the current trial.

5.3.2 Lipid and Moisture Analysis

The lipid and moisture contents of the thirty fish from each cage were determined (table 5.3.2). From the list of individual lipid contents twenty fish were selected from each cage so that groups of five fish (one from each cage) of similar lipid contents could be made for taste panelling. This was essential for the taste panelling part of the trial following the results shown in chapter 3 of this thesis, which demonstrated the importance of lipid content on some of the eating quality parameters of cooked fish.

Table 5.3.2 Moisture and lipid contents of the white muscle samples of fish from each of the five cages (n=30).

	Moisture (%)	s.e.m.	Lipid (%)	s.e.m.
Cage 1	71.47	0.247	5.04	0.290
Cage 2	71.98	0.264	4.31	0.299
Cage 3	70.33	0.263	5.33	0.388
Cage 4	71.04	0.208	5.51	0.210
Cage 5	71.25	0.390	5.39	0.429

The distribution of lipid contents throughout the entire group of 150 fish is shown in figure 5.3.2. The distribution appears to be centred around a mean of approximately 5% lipid, with one extremely high lipid content (12.1%) and one very low (0.3%). From the results of the lipid analyses twenty fish were selected from each dietary group according to their lipid contents. This resulted in twenty groups of five fish matched as closely as possible for their lipid content.

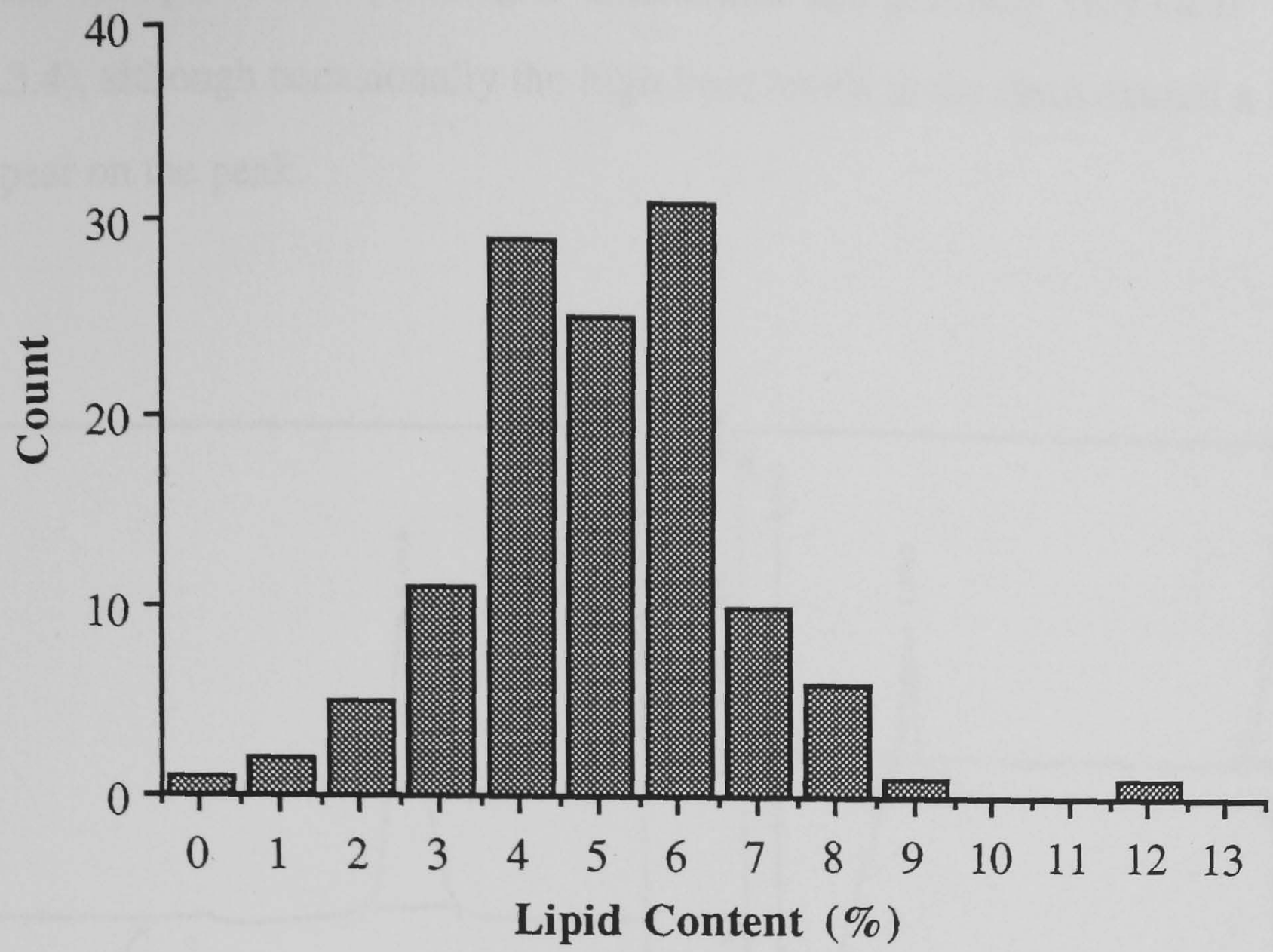

Figure 5.3.2 White muscle lipid content distribution across the five cages.

5.3.3 Astaxanthin Analysis

The astaxanthin concentration of the one hundred selected fish was determined using HPLC. Samples for astaxanthin analysis were taken after slaughter and after 6 and 12 days of storage of the fillets on ice. Although the extraction protocol was straightforward to implement, pressures on the chromatography equipment and the large number of samples involved in this experiment meant that the samples had to be stored for a period of up to 2 months after extraction before they could be analysed for the pigment. This led to eleven samples being lost owing to leaking vials and subsequent evaporation.

The peak on the chromatogram of the standard appeared between 5 and 6 minutes after injection (figure 5.3.3) — the time depending on the exact flow rate through the column. The chromatogram resulting from the samples was very clear, with only two peaks appearing. The first was probably canthaxanthin, but unfortunately no standard was available when the samples were being run to determine whether this assumption

was correct. The peak corresponding to astaxanthin was generally very clear (figure 5.3.4), although occasionally the high lipid levels in the flesh caused a long tail to appear on the peak.

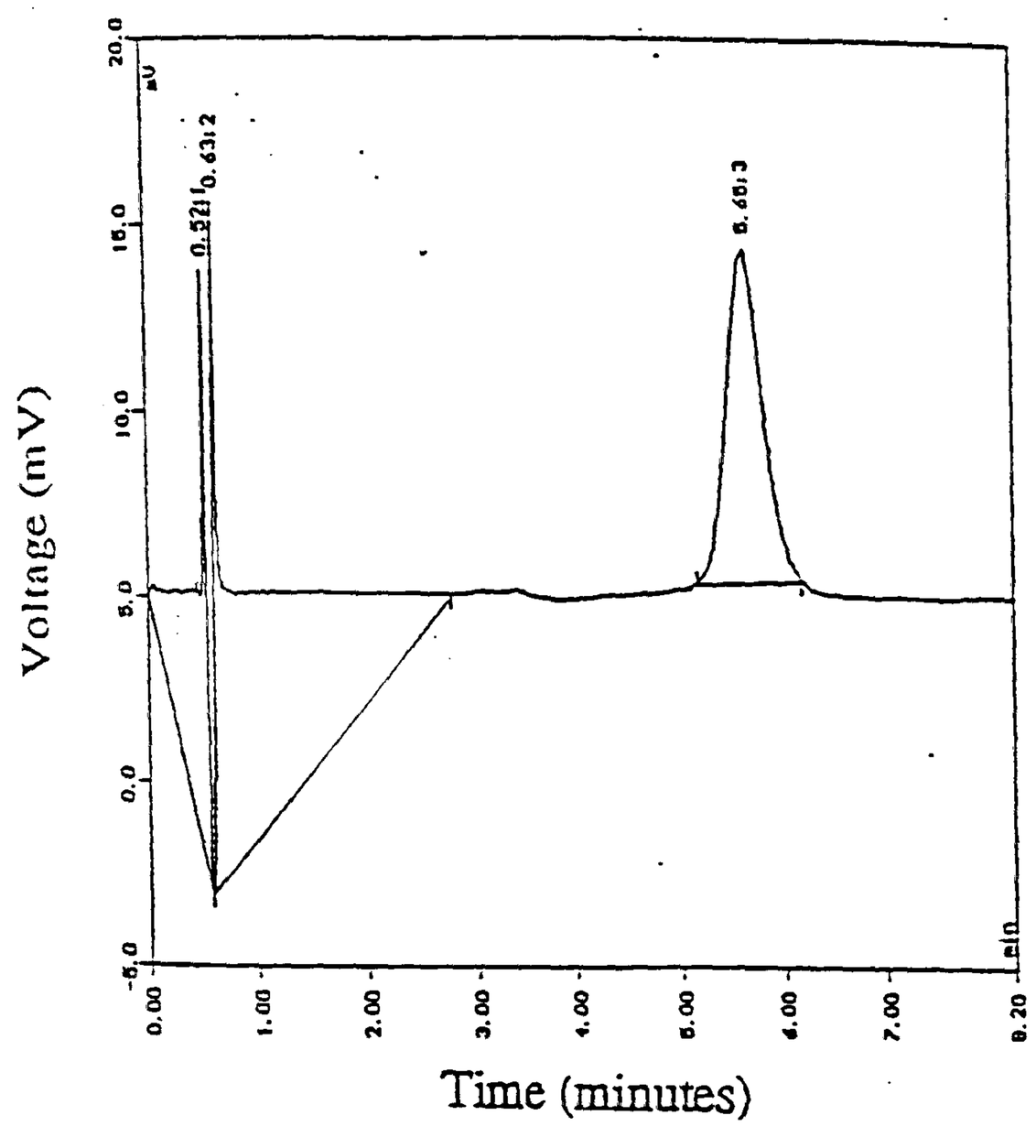

Figure 5.3.3 Chromatogram of a standard solution of astaxanthin.

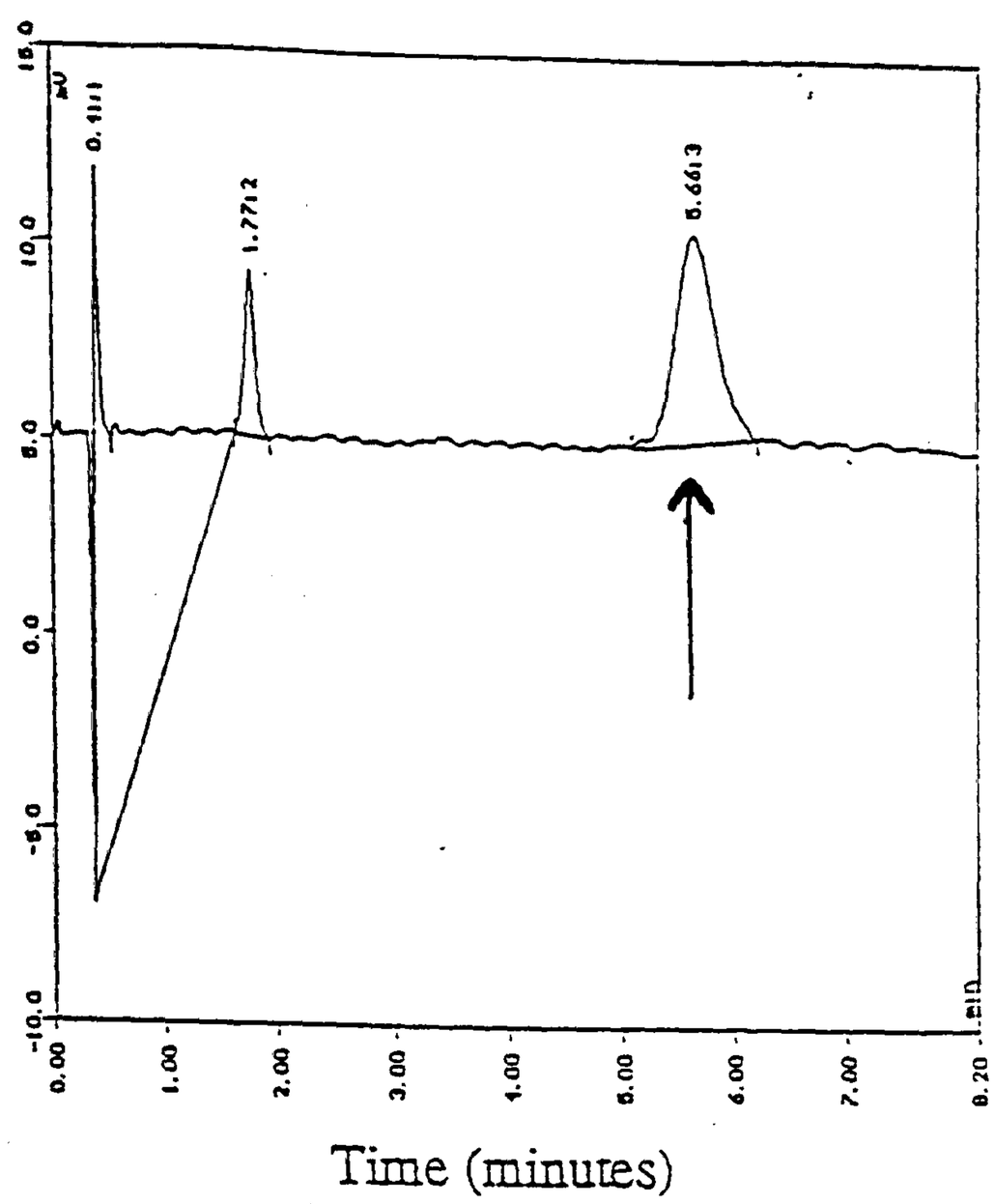

Figure 5.3.4 Chromatogram of a sample of astaxanthin (arrowed).

The mean astaxanthin concentrations of the twenty fish from each cage are shown in table 5.3.3. The initial differences in mean astaxanthin concentration between the fish in each cage are highlighted in figure 5.3.5. No statistical analysis was carried out between the diets as *n* was equal to one — *i.e.* the number of cages fed each diet (the fish were not statistically independent).

Table 5.3.3 Changes in mean astaxanthin concentrations (ppm) in the white muscle of the fish from each cage.

	0 days		6 days		12 Days	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
Cage 1	4.71	0.309	4.03	0.249	3.94	0.383
Cage 2	3.86	0.475	4.04	0.334	4.80	0.399
Cage 3	4.56	0.393	4.55	0.268	4.58	0.406
Cage 4	4.43	0.323	4.22	0.308	4.66	0.281
Cage 5	4.91	0.293	4.56	0.281	4.43	0.285

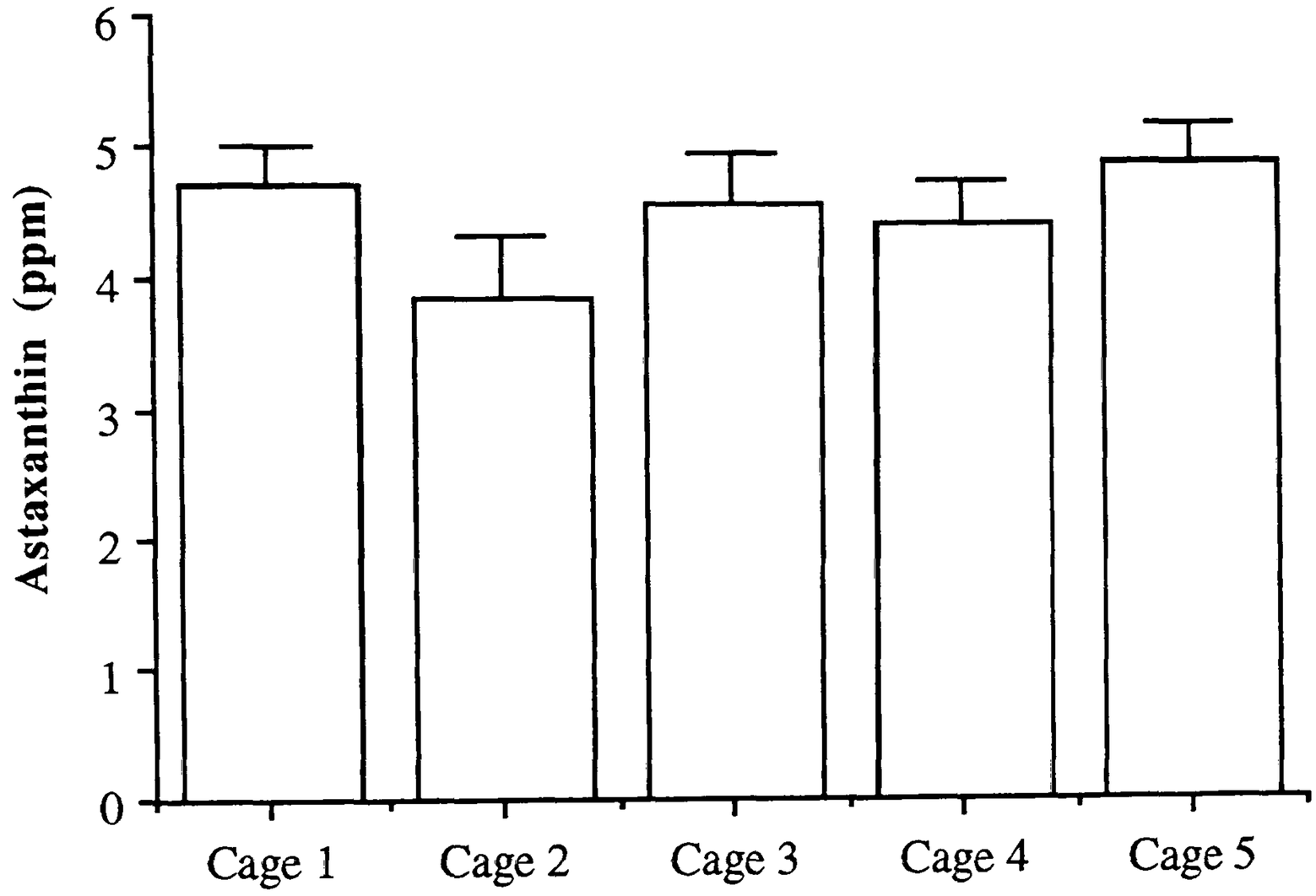

Figure 5.3.5 Mean astaxanthin concentration (+ s.e.m.) of the white muscle of the fish from each cage after slaughter.

The overall mean level of astaxanthin after slaughter was 4.51 ± 0.161 ppm. After storage the overall mean was 4.49 ± 0.161 ppm. This showed that there was no significant loss of pigment during twelve days of storage on ice (two-tailed, paired t-test, t = -358, d.f. = 59, $p>0.05$).

5.3.4 Vitamin C Analysis

The ascorbic acid concentration content of the one hundred selected fish was determined using HPLC. Only samples taken after slaughter were analysed for ascorbic acid. A lot of difficulty was experienced with this assay as a precipitate tended to form in the extracted samples during storage prior to the chromatography. Owing to pressures of time, it was not possible to extract the samples and immediately analyse them for the vitamin content. They had to be stored as extracted samples for up to 2 months as this was felt to be preferable to storing them unextracted owing to the labile nature of ascorbic acid. Formation of the precipitate during this storage led to twenty-one of the samples being rendered useless for analytical purposes. The precipitate caused the appearance of large peaks on the chromatogram, often masking the ascorbic acid peak and making it impossible to determine the ascorbic acid content of the sample.

When the precipitate had not formed the chromatogram was fairly clear, with the ascorbic acid peak clearly discernible from the chromatogram of the standard (figure 5.3.6), although a lot of other peaks were detected at this wavelength (figure 5.3.7). The mean ascorbic acid concentration of the fish from each cage was determined (table 5.3.4), but as with the astaxanthin no statistical analyses were carried out at this point. However, a study of the means shows that the different cages had different means and that the diets containing low levels of ascorbic acid had produced fish with lower levels of ascorbic acid in their flesh (figure 5.3.8).

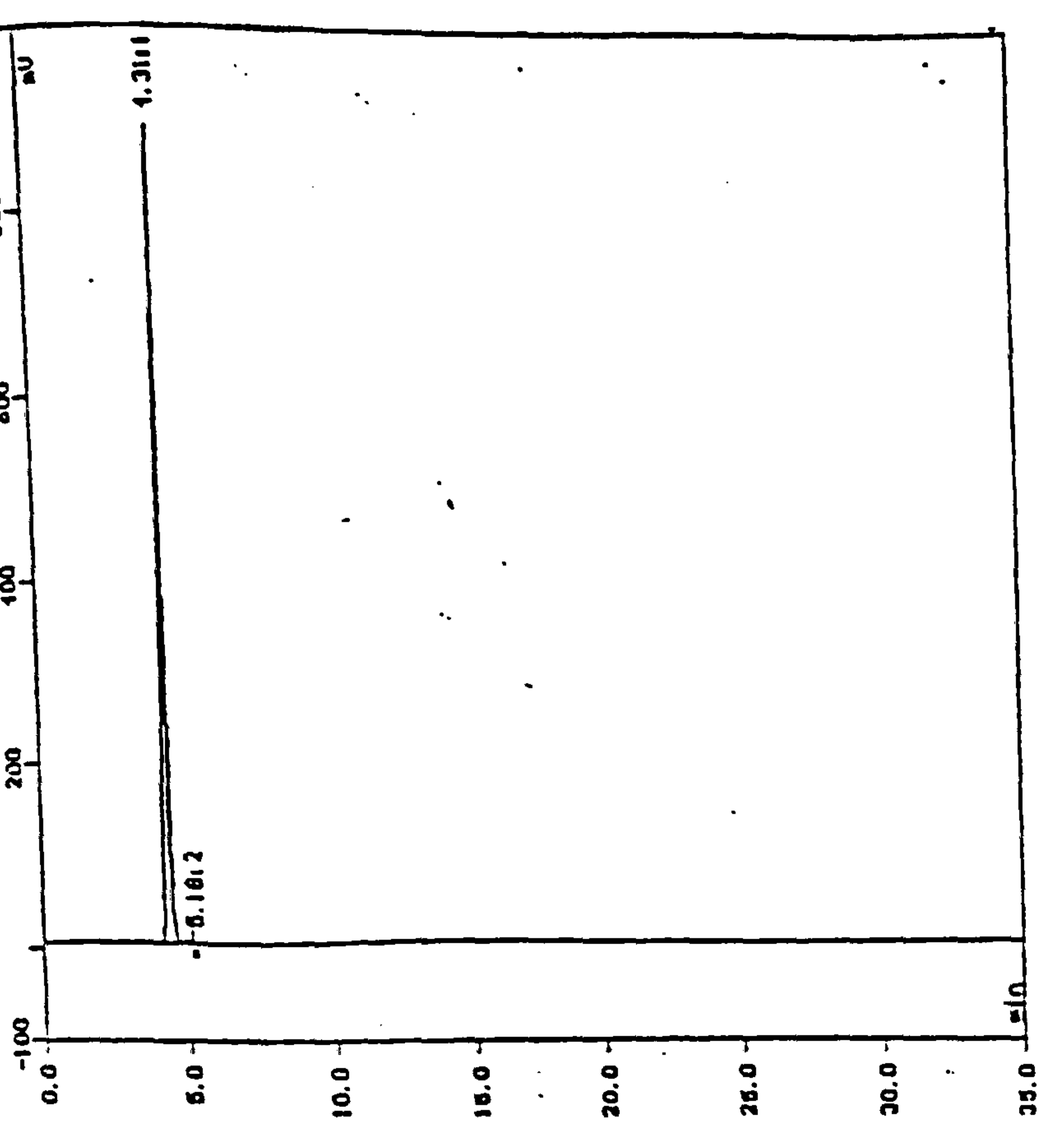

Figure 5.3.6 Chromatogram of a standard solution of ascorbic acid.

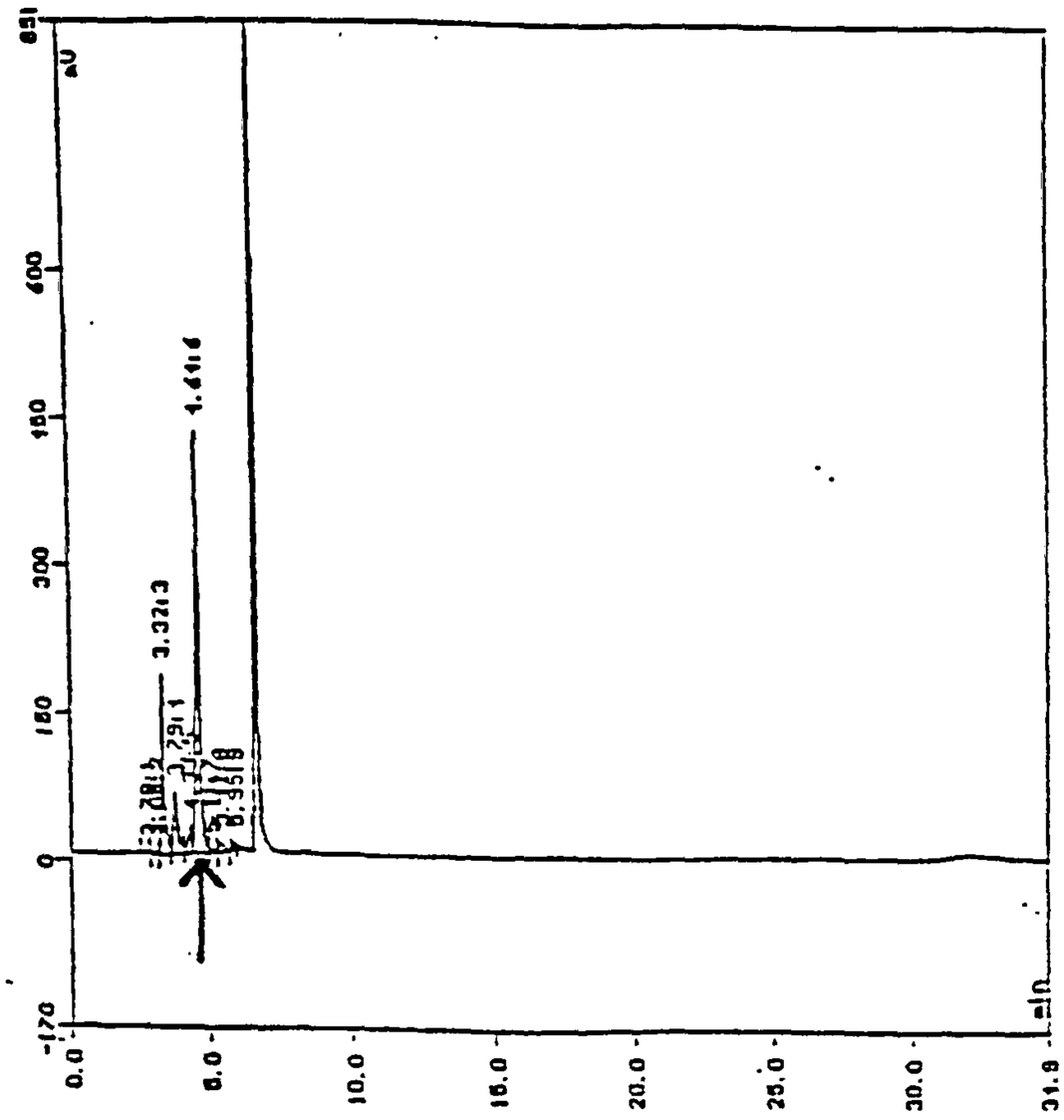

Figure 5.3.7 Chromatogram of a sample of ascorbic acid (arrowed).

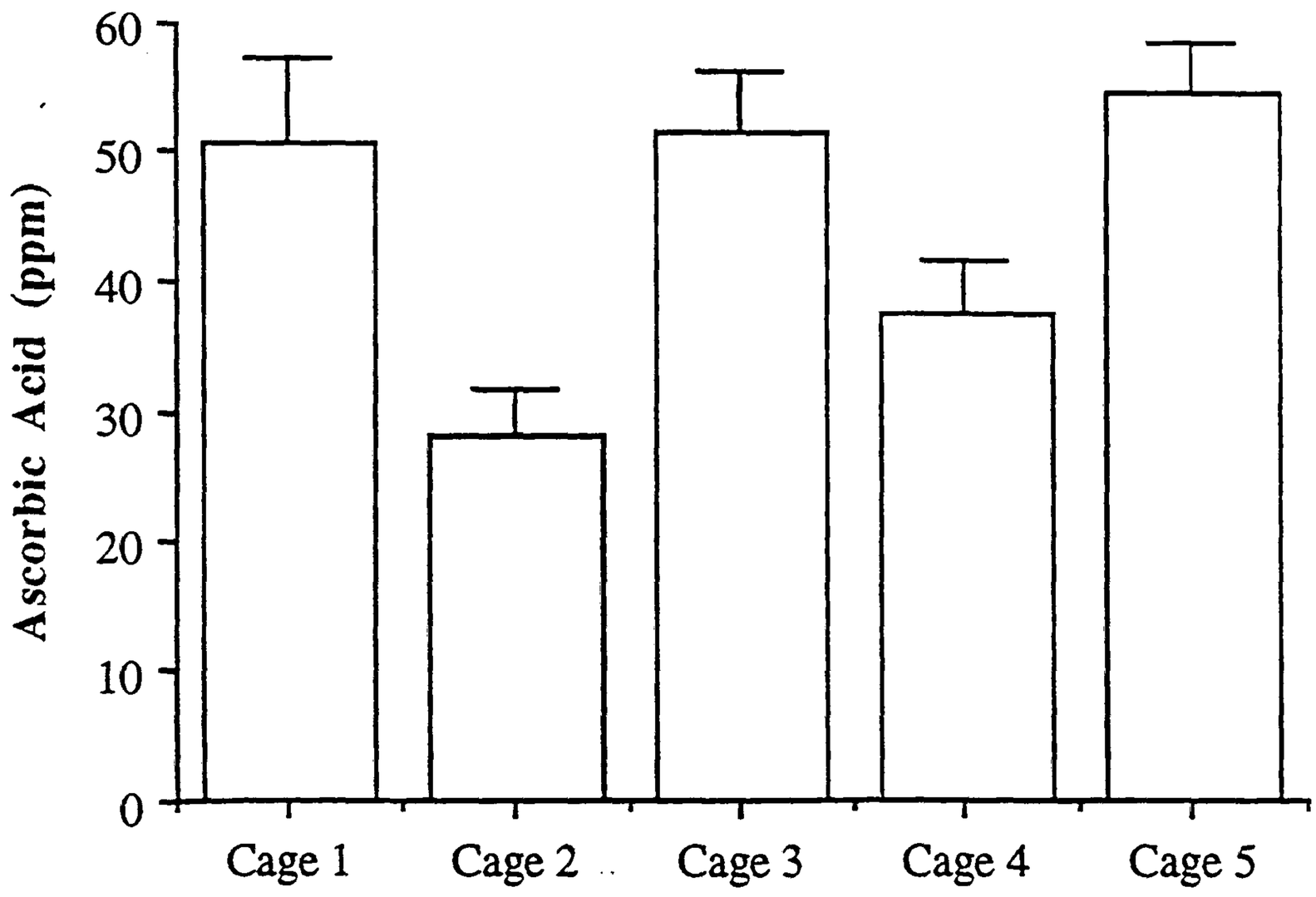

Figure 5.3.8 Mean ascorbic acid concentration (+ s.e.m.) of the white muscle of the fish from each cage after slaughter.

Table: 5.3.4 Mean white muscle ascorbic acid concentration (ppm) of the fish from each cage (± s.e.m.) after slaughter.

	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5
Ascorbic Acid Conc.	50.79 (6.483)	28.04 (3.496)	51.71 (4.661)	37.61 (4.174)	54.93 (3.835)

The effect of the ascorbic acid on the astaxanthin concentration was then investigated. No correlation was found between ascorbic acid and astaxanthin concentration after slaughter (figure 5.3.9) and there was no correlation with the ascorbic acid concentration after slaughter and the percentage loss of astaxanthin during 12 days storage on ice (figure 5.3.10).

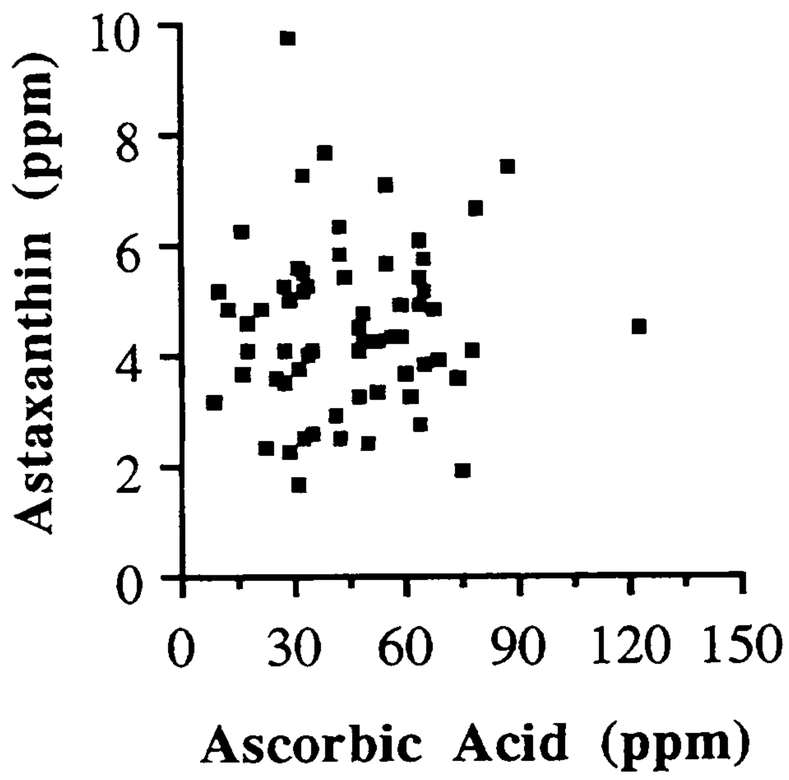

Figure 5.3.9 Effect of ascorbic acid on astaxanthin concentration after slaughter.

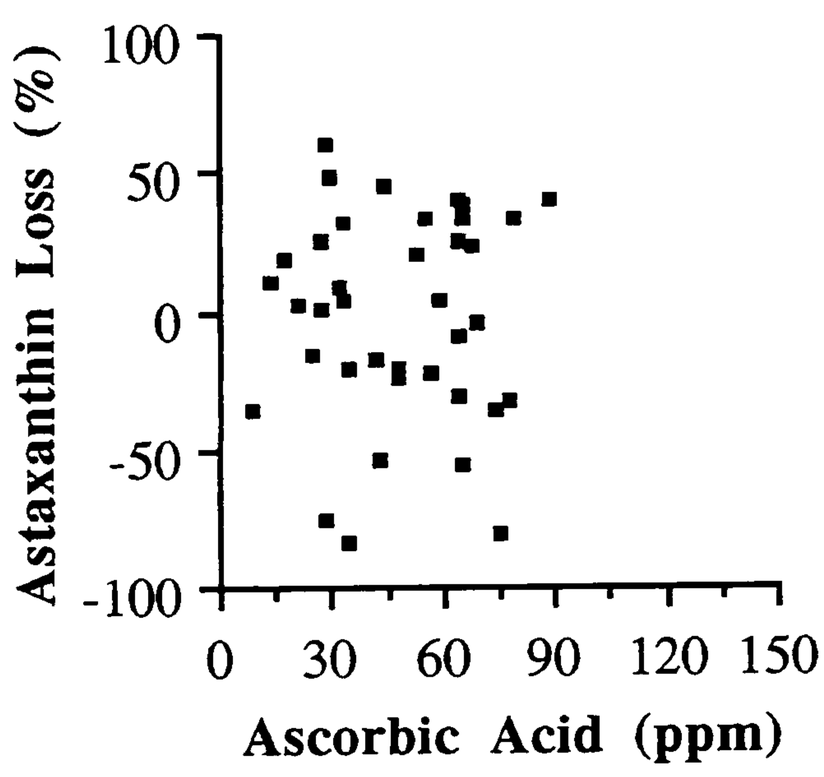

Figure 5.3.10 Effect of ascorbic acid on change in astaxanthin during storage.

5.3.5 Vitamin E Analysis

The extraction of the α-tocopherol was straight forward, but very time consuming. As only a few samples per day could be extracted the process was long and so only the samples taken immediately after slaughter were extracted. Although the other sample points would have given information on the loss of α-tocopherol during

storage on ice, the losses occurring during the frozen storage of the samples prior to extraction would have lead to confusing results. Again pressures on the use of the chromatography equipment led to long storage periods of the extracted samples prior to analysis, but only four samples were lost owing to evaporation or leakage.

As with the ascorbic acid chromatograms there were many other peaks visible on the sample chromatogram at this wavelength (figure 5.3.12). However, it was straight-forward to determine which was the α-tocopherol peak from the standard (figure 5.3.11) and thus calculate its area. From this the mean α-tocopherol concentrations of the samples from each cage were determined (table 5.3.5). As with the astaxanthin, no statistical analysis was carried out at this point, but it was apparent that the fish fed the diets with low levels of α-tocopherol had low levels of α-tocopherol in their flesh and *vice versa* (figure 5.3.13).

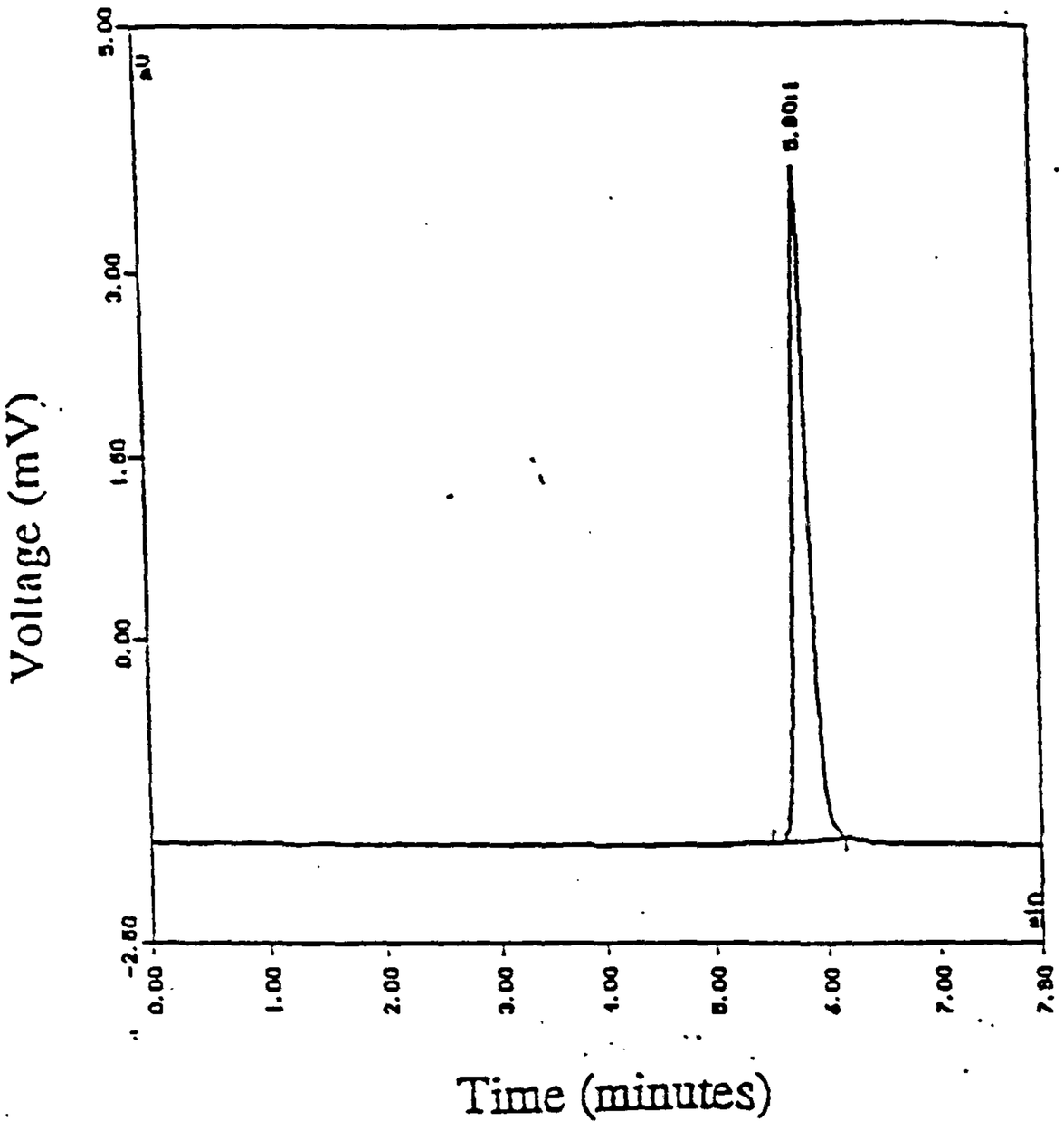

Figure 5.3.11 Chromatogram of a standard solution of α-tocopherol.

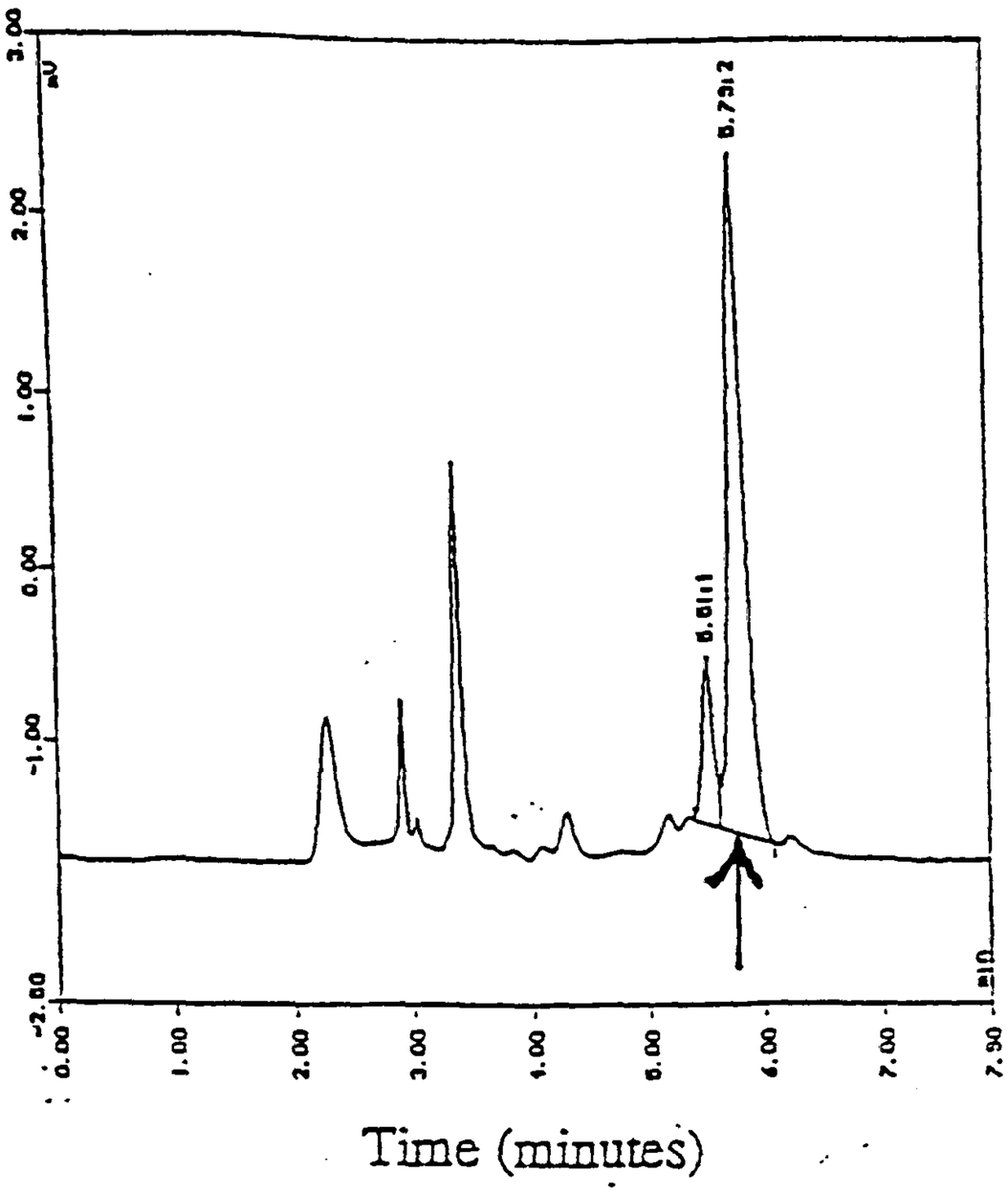

Figure 5.3.12 Chromatogram of a sample of α-tocopherol (arrowed).

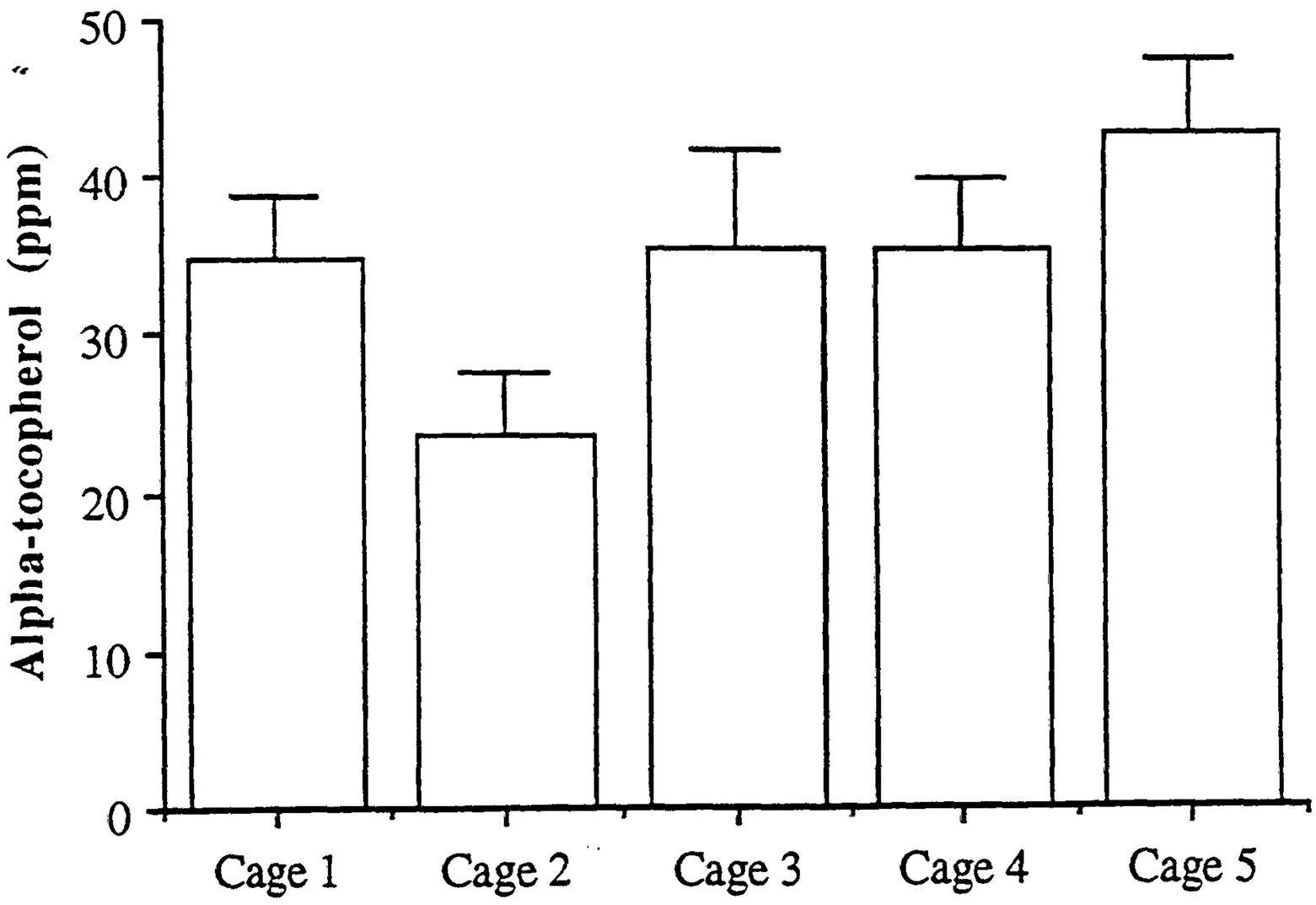

Figure 5.3.13 Mean α-tocopherol concentration (± s.e.m.) of the white muscle of the fish from each cage after slaughter.

Table 5.3.5 Mean α-tocopherol concentrations (ppm) of the white muscle of fish from each cage (± s.e.m.) after slaughter.

	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5
α-Tocopherol Conc.	34.75 (3.934)	23.60 (3.911)	35.58 (6.100)	35.56 (4.380)	43.05 (4.826)

The effect of the α-tocopherol concentration after slaughter on the astaxanthin concentration at that point was investigated (figure 5.3.14), but there was no correlation between the two. There was also no effect of α-tocopherol on astaxanthin loss during the period of storage on ice (figure 5.3.15).

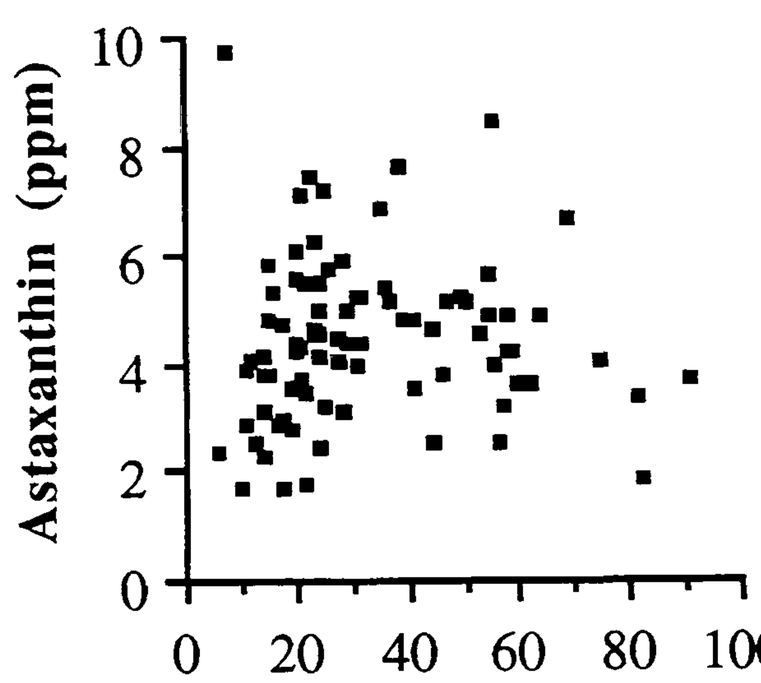

Figure 5.3.14 Effect of α-tocopherol on astaxanthin concentration at slaughter.

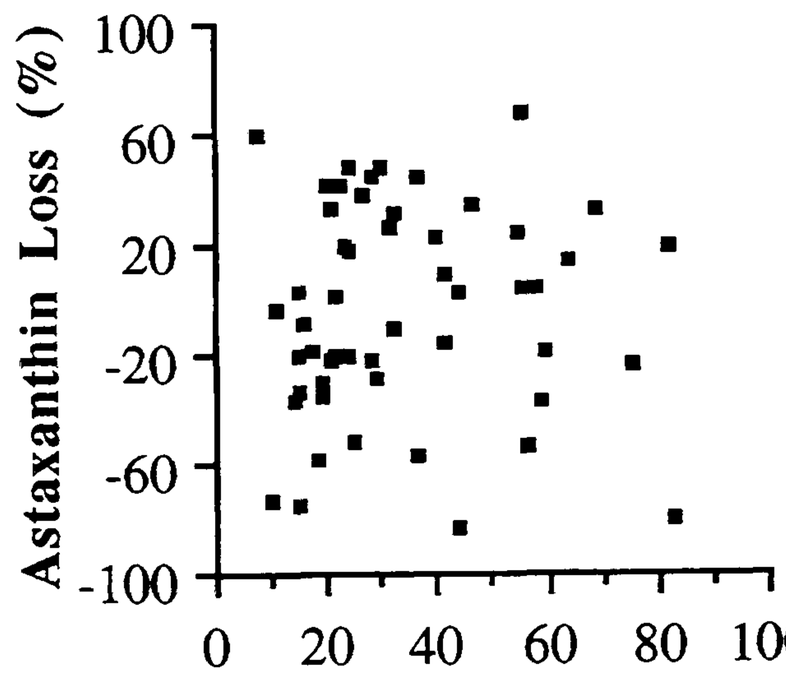

Figure 5.3.15 Effect of α-tocopherol on astaxanthin loss during storage.

5.3.6 Eating Quality

During the training sessions, the taste panellists agreed between themselves to use the same attributes to describe the cooked fish, both before and after storage on ice, as were used in chapter 3 of this thesis. These attributes are summarised in table 5.3.6 and table 5.3.7.

Table 5.3.6 Texture attributes.

Texture on Cutting	*Firm*	The perceived force to cut the sample with a knife
	Disintegration	The degree to which the sample breaks up during cutting
Texture on	*Slimy*	Moist, slippery texture on the sample.
First Bite and Chewing	*Firm*	The perceived force required to compress and chew the sample.
	Moist	The perceived degree of oil / water in the sample during chewing.
	Sticky	Degree to which the sample stuck to the teeth and palate during chewing.
	Gelatinous	Amount of gelatinous jelly texture.
	Chewy	The total perceived effort required to prepare the sample to a state ready for swallowing.
	Fibrous	Degree to which fibres could be detected during chewing.
	Cohesive	Degree to which the sample sticks together before swallowing.
	Dissolubility	Degree to which the sample melts in the mouth.

Table 5.3.7 Flavour attributes.

Flavour	*Fishy*	Amount of fish flavour
On Chewing	*Bitter*	Amount of bitter flavour
	Creamy	Flavour associated with cream.
	Seaweed	Flavour associated with seaweed.
	Oily	Taste associated with liquid fat.
	Sour	Acidic flavour.
	Sweet	Taste associated with sugary foods.
	Metallic	Taste of metal.
	Earthy	Taste associated with mud.
	Stale	Taste associated with off flavours.
	Salty	Taste associated with salt.

The mean ratings for each fillet were determined and the correlation with the astaxanthin, ascorbic acid and α-tocopherol concentrations in the white muscle determined for both the fresh fillets and the fillets which had been stored on ice (table 5.3.8). Very few significant correlations were found and those that were found did not account for much of the variance in the ratings — the square of the correlation coefficient *r* gives the degree of variance accounted for (Zolman, 1993). In other words other factors had a greater effect on the ratings than the levels of these three anti-oxidants after slaughter.

Table 5.3.8 Correlation coefficients, *r*, of the texture and flavour ratings of fresh and stored fish with astaxanthin ($n = 89$), ascorbic acid ($n = 71$) and α-tocopherol ($n = 94$) concentrations after slaughter. Levels of significance are indicated by * ($p<0.05$) and ** ($p<0.01$).

	Astaxanthin Conc.		Ascorbic Acid Conc.		α-Tocopherol Conc.	
	Fresh	Stored	Fresh	Stored	Fresh	Stored
Firm on Cut	0.113	-0.092	0.201	0.055	0.034	0.041
Disintegration	-0.141	0.140	-0.100	0.031	0.064	-0.019
Slimy	-0.031	-0.116	-0.145	-0.064	-0.137	-0.017
Firm on Chew	0.093	-0.026	0.087	0.146	0.033	0.194
Moist	-0.077	-0.048	-0.076	-0.098	-0.135	-0.002
Sticky	0.141	-0.188	-0.160	0.191	0.160	0.084
Gelatinous	-0.008	-0.005	-0.212	-0.095	-0.168	0.068
Chewy	0.176	0.079	0.132	0.025	0.065	0.217*
Fibrous	-0.071	-0.165	-0.154	0.143	0.132	0.171
Cohesive	0.199	-0.004	0.122	0.084	0.009	-0.051
Dissolubility	-0.101	-0.041	-0.047	-0.098	0.169	0.098
Fishy	0.188	-0.039	-0.137	0.012	0.030	0.015
Bitter	0.027	0.120	-0.044	0.249	-0.051	-0.028
Creamy	0.058	-0.026	-0.026	-0.310**	0.082	-0.099
Seaweed	-0.183	0.131	-0.158	0.265*	-0.170	0.155
Oily	0.080	-0.124	0.017	0.016	-0.215*	0.020
Sour	-0.018	0.067	0.017	0.343	0.041	0.141
Sweet	0.020	-0.159	-0.069	-0.112	0.233*	0.025
Metallic	-0.251*	0.019	0.108	0.141	-0.216*	0.123
Earthy	-0.002	0.089	-0.076	0.046	-0.023	0.065
Stale	0.021	-0.020	0.069	0.015	-0.121	0.165
Salty	0.147	-0.301*	0.157	-0.025	0.059	-0.124
Overall Flavour	0.181	-0.115	-0.091	-0.103	0.106	-0.064
Overall Liking	0.195	-0.115	-0.049	-0.116	0.140	-0.105

5.3.6.1 Effects of Storage on Attributes

The mean ratings of each attribute for each fillet were known for both fresh and stored fillets. Therefore it was possible to determine the effect of storage on ice on the eating quality of the flesh. A two-tailed t-test was carried out on the pairs of mean values (table 5.3.9) and the direction of the changes highlighted.

Table 5.3.9 Effect of storage on the texture and flavour attribute ratings. t Values are from the two-tailed paired t-test, and the degree of significance is shown in the final column.

	Fresh Ratings (± s.e.m.)	Stored Ratings (± s.e.m.)	Change	t Value	Signif. (*p*)
Firm on Cut	25.40 (0.478)	25.13 (0.568)	None	0.406	ns
Disintegration	44.25 (0.821)	36.28 (0.773)	Decrease	7.241	<0.001
Slimy	12.06 (0.519)	14.27 (0.563)	Increase	-3.844	<0.001
Firm on Chew	25.11 (0.754)	26.99 (0.565)	Increase	-2.261	<0.05
Moist	41.84 (0.655)	43.42 (0.540)	Increase	-2.344	<0.05
Sticky	21.58 (0.394)	23.26 (0.584)	Increase	-2.581	<0.05
Gelatinous	3.80 (0.342)	6.54 (0.391)	Increase	-5.870	<0.001
Chewy	30.81 (0.667)	30.08 (0.553)	None	0.873	ns
Fibrous	21.06 (0.464)	21.99 (0.312)	None	-1.717	ns
Cohesive	40.93 (0.488)	39.38 (0.545)	Decrease	2.290	<0.05
Dissolubility	18.81 (0.496)	22.75 (0.611)	Increase	-4.827	<0.001
Fishy	30.10 (0.406)	34.77 (0.412)	Increase	-8.082	<0.001
Bitter	10.20 (0.485)	16.53 (0.634)	Increase	-8.575	<0.001
Creamy	18.72 (0.543)	14.32 (0.573)	Decrease	6.449	<0.001
Seaweed	14.65 (0.405)	15.46 (0.499)	None	-1.335	ns
Oily	14.62 (0.429)	18.53 (0.387)	Increase	-6.207	<0.001
Sour	6.61 (0.314)	9.07 (0.407)	Increase	-5.387	<0.001
Sweet	9.78 (0.364)	9.51 (0.422)	None	0.571	ns
Metallic	4.62 (0.386)	8.72 (0.463)	Increase	-6.616	<0.001
Earthy	15.62 (0.475)	13.39 (0.455)	Decrease	3.267	<0.01
Stale	15.12 (0.537)	18.33 (0.546)	Increase	-4.507	<0.001
Salty	4.29 (0.177)	6.91 (0.190)	Increase	-9.410	<0.001
Overall Flavour	33.21 (0.728)	27.97 (0.696)	Decrease	6.049	<0.001
Overall Liking	35.16 (0.762)	29.93 (0.701)	Decrease	5.908	<0.001

The effects of storage on the attributes are easily seen from a radar plot of the means. The changes in texture during storage show that with storage the flesh became significantly more likely to break up (higher *disintegration* and *dissolubility*) during chewing and was more *slimy* and *gelatinous*. (figure 5.3.16). *Firm* texture on chewing decreased and the flesh became less *cohesive* ($p<0.05$).

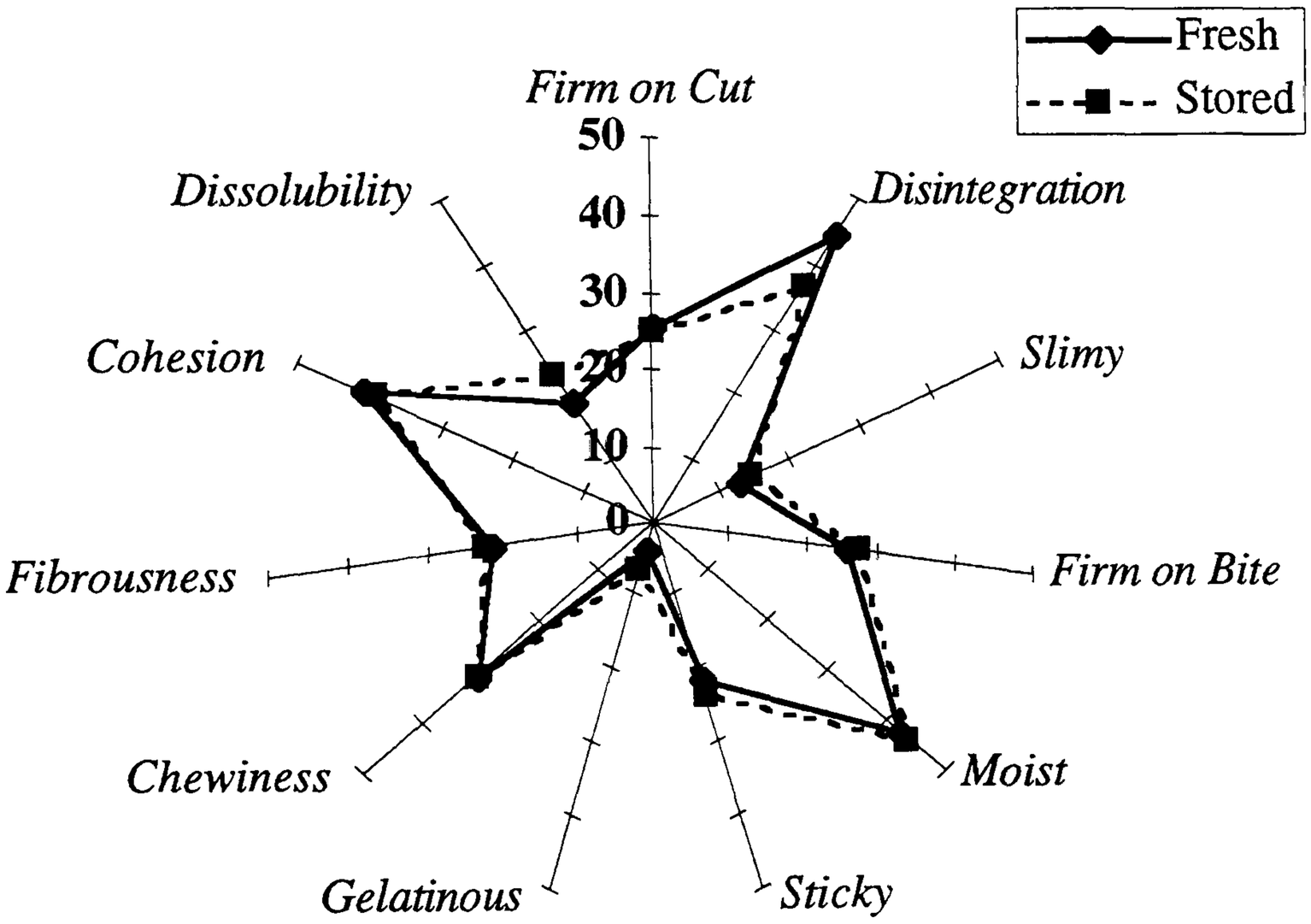

Figure 5.3.16 Radar plot of the changes in texture attribute ratings with storage.

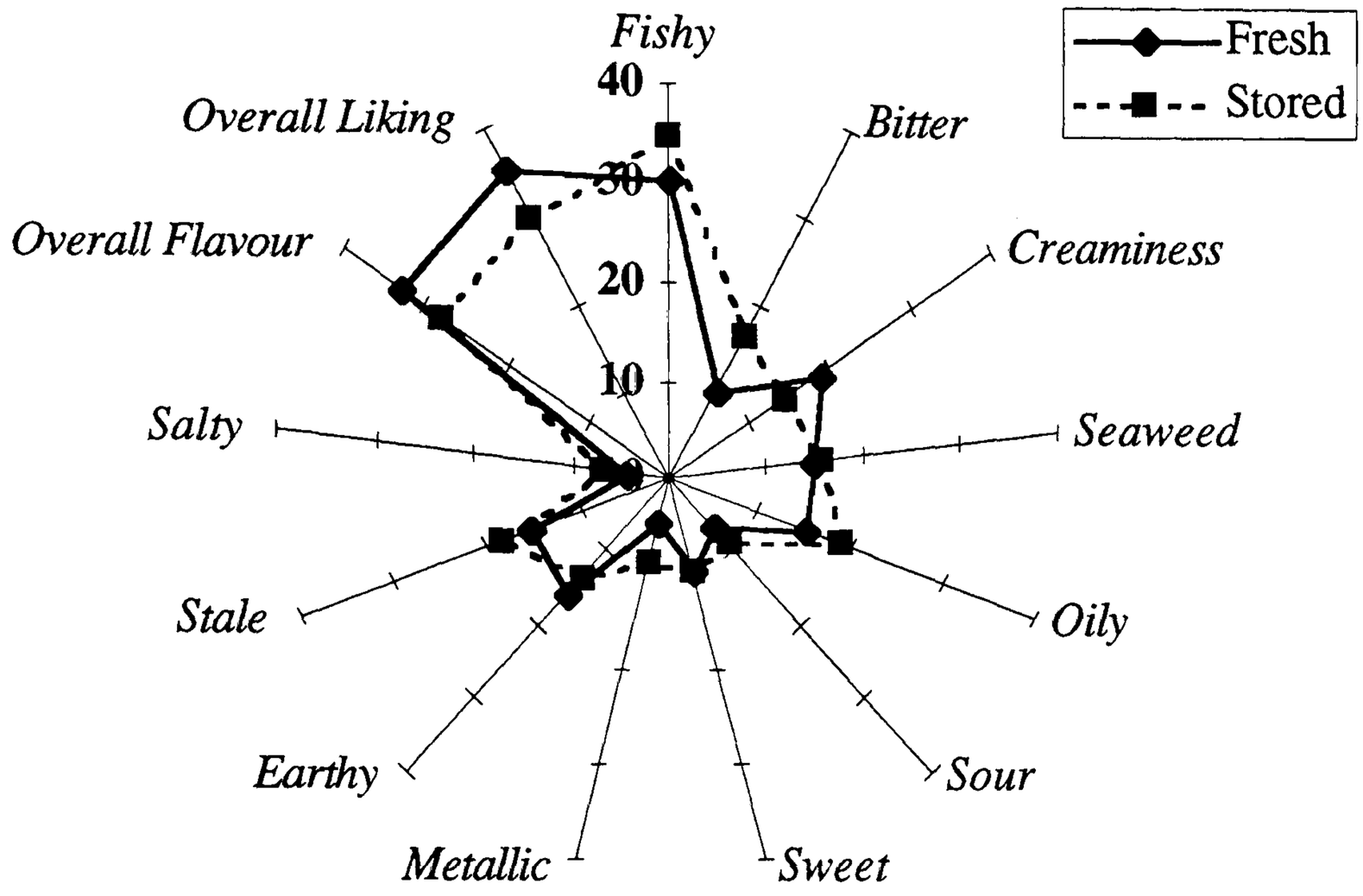

Figure 5.3.17 Radar plot of the changes in flavour attribute ratings with storage.

The flavours associated with stored fish were sharper tasting (increased *bitter, sour* and *metallic*, with lower *creaminess*) and had more *stale* flavour (figure 5.3.17). *Fishy* flavour increased, as did *oily* and *salty* flavours ($p<0.001$). There was, however, no change in *sweet* flavour ($p>0.05$) and there was a decrease in *earthy* flavour ($p<0.05$). Overall flavour and overall liking decreased significantly with storage ($p<0.001$).

5.3.6.2 Factors Affecting Individual Attributes

A correlation matrix of the mean results for each fish was drawn up, correlating all of the texture and flavour attributes and the chemical parameters. From this matrix elementary linkage analysis (ELA) was carried out to determine where important relationships between attributes and the chemical composition of the fish lay. Appendix 3 describes the structure of the analysis. From the ELA four clusters were determined (figures 5.3.18 to 5.3.21).

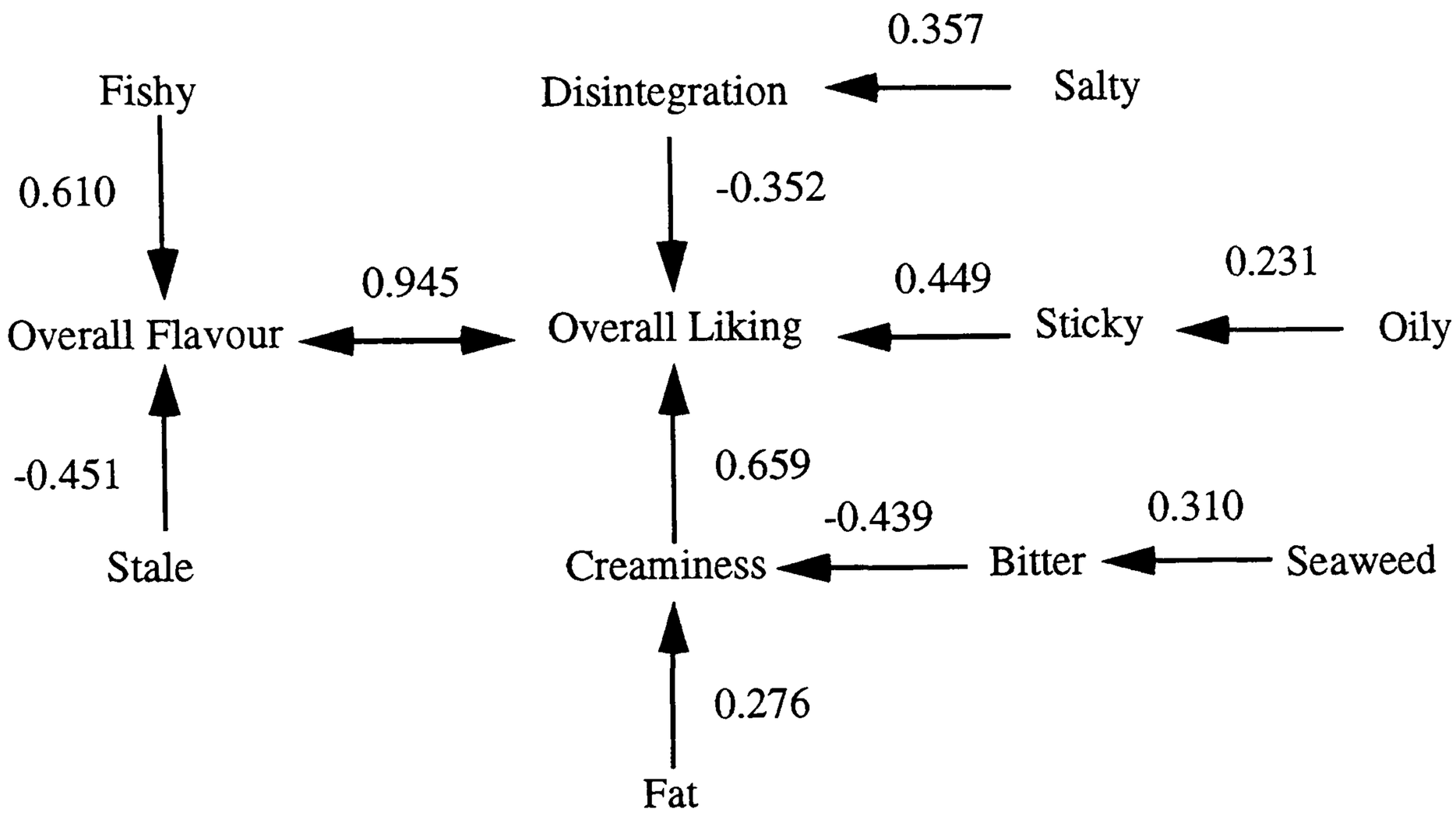

Figure 5.3.18 Cluster 1 of the attributes and composition parameters of the fresh, cooked salmon.

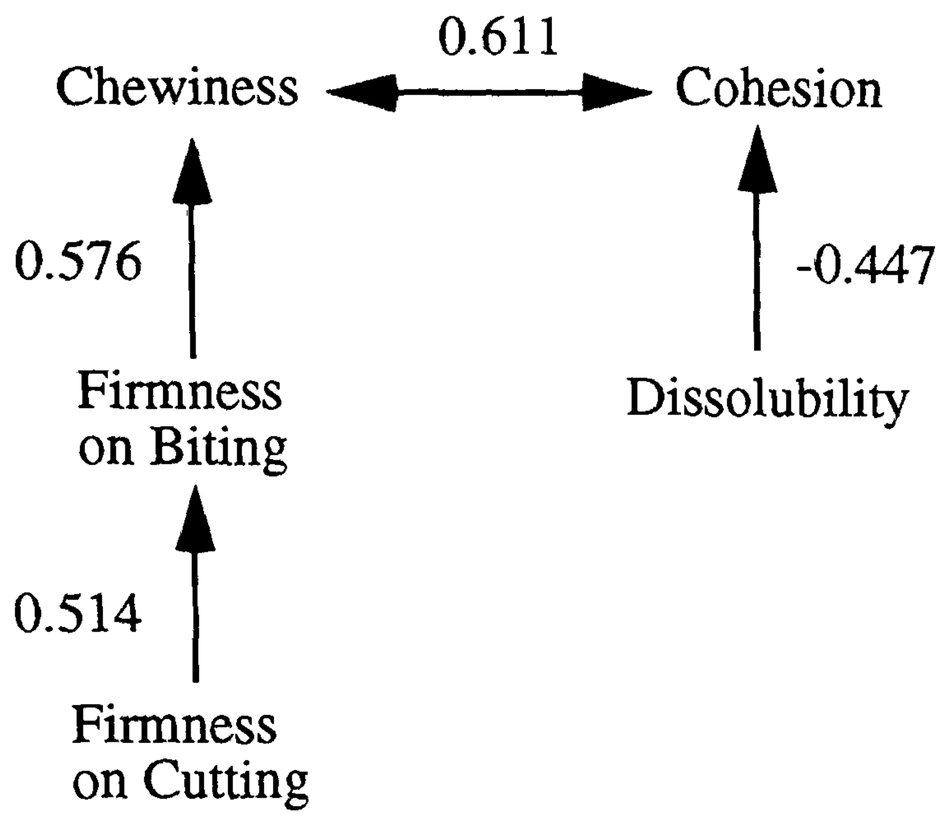

Figure 5.3.19 Cluster 2 of the fresh, cooked salmon.

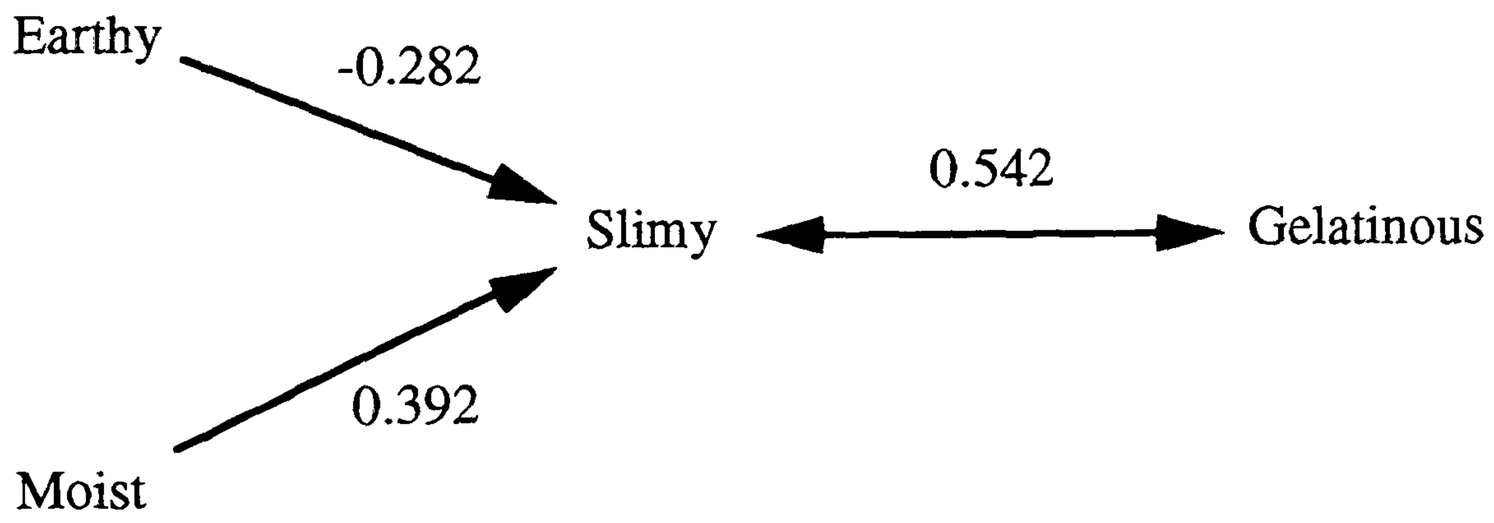

Figure 5.3.20 Cluster 3 of the fresh, cooked salmon

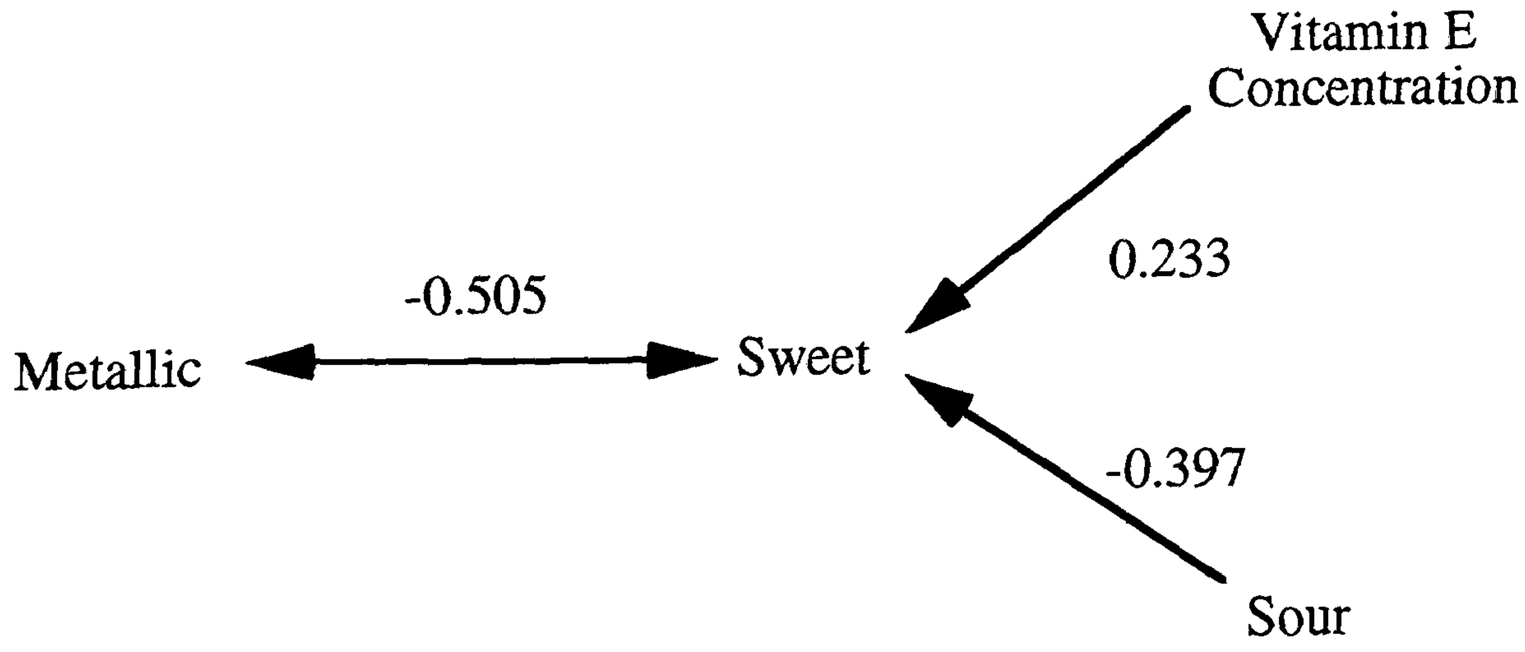

Figure 5.3.21 Cluster 4 of the fresh, cooked salmon.

5.4 Discussion

5.4.1 Growth

The sea lice infestation obviously had a large effect on the fish, causing loss of weight over the last six weeks of the trial. As the lice infestation affected all of the cages, the effects on the growth of the fish were assumed to be the same for all cages (figure 5.3.1). However, the fish in cage 2 were significantly smaller than all other fish, except for those in cage 5, at the end of the trial. The diets fed to these fish had slightly lower oil levels than those fed to the other cages, which could have resulted in the slower growth. Alternatively it could have been because of greater effects by the sea lice on the fish in cage 2. The fish in this cage received lower levels of ascorbic acid and α-tocopherol in their diet and these vitamins are known to increase resistance to infection, especially the ascorbic acid (reviewed by Gabaudan *et al.*, 1990). The levels of the vitamins were below those recommended for standard feeding (Steffens, 1989) and it is recommended that these levels be increased during times of stress or infection (Gabaudan *et al.*, 1990). However, the duration of the feeding was relatively short, so it was hoped that the fish would not become immuno-challenged during the trial.

The effect of some fish not feeding was also apparent from the lipid content of the fish (figure 5.3.2). The lipid content of the white muscle of some fish was very low (less than 1%), which may have been a result of fasting (Wiseman, 1993). The mean lipid contents of the fish from each cage also show that the fish from cage 2 had lower lipid contents (table 5.3.2). This supports the supposition that these fish were affected more than those in other cages by the sea lice infestation, or some other unknown stress, as the difference in oil level between the diets was too small to create such a difference in the short period of the trial.

5.4.2 Astaxanthin and Vitamins

The levels of vitamins in the diets for this trial resulted in a wide variation in the levels of the vitamins in the flesh. Although it was not correct to statistically analyse the differences between the fish fed each diet, as each diet was only fed to one cage, looking at the mean levels it can be seen that the diets may have had an effect (figures 5.3.8 and 5.3.13). This supports the findings of Frigg *et al.* (1990) and Sigurgisladottir *et al.* (1994) who showed that feeding diets with different levels of tocopherols resulted in a significant difference between the muscle tocopherol contents. This difference was found by Sigurgisladottir *et al.* (1994) even with very low numbers of fish ($n = 3$) — also using just one tank per diet.

Difficulties were experienced with the assays for the vitamins. These resulted in a number of samples being lost and thus the power of some of the analytical tests was reduced. Further, the difficulties with the extractions and chromatography may have resulted in some spurious results. This would have led to further problems with the attempts to find correlations between the levels of the vitamins and the astaxanthin levels.

Investigation of the astaxanthin content of the fish showed that there was an apparent difference between the fish fed some of the diets (figure 5.3.5). The fish fed diet 2 had the lowest levels of astaxanthin and those fed diet 5 had the highest after slaughter. This would infer that the increased levels of vitamins in the diet resulted in increased deposition of astaxanthin in the flesh of the fish, but after 12 days of storage this difference had been lost (table 5.3.3). However, neither Torrissen (1985) nor Sigurgisladottir *et al* (1994) found an effect resulting from an increase in the level of tocopherols in the diet on the deposition of astaxanthin in the muscle, although it should be noted that Sigurgisladottir *et al* (1994) used very low numbers of fish

($n = 3$) with only one tank per dietary treatment and so their conclusions should be treated with caution.

During the storage period there was a wide discrepancy in the amount of astaxanthin lost from the samples. Some fish showed a marked decrease in their astaxanthin concentration and others showed an increase in the concentration. This increase could have been caused by the loss of moisture from the flesh during storage on ice. It could also have been caused by loss of moisture during the frozen storage of the samples. Some samples showed severe freezer burn, a condition caused by the loss of moisture from the sample during both the freezing process and the frozen storage to which fish flesh is particularly susceptible (Connell, 1995). During freezer burn moisture is drawn out of the flesh, ultimately leaving it very dry and brittle. As the flesh normally was about 70% moisture (table 5.3.2) losses from freezer burn of this would have resulted in the large increases in the concentration of astaxanthin observed in some samples.

Looking at the overall mean astaxanthin concentrations there was no change in concentration during the storage period. This supports the findings of Chen *et al.* (1984) who found no change in 14 days of storage of trout fillets in air in the dark at 1-2˚C. In contrast, short periods of frozen storage seem to give a large decrease in astaxanthin concentrations (Chen *et al.*, 1984; Bjerkeng and Johnsen, 1995).

No correlation was found between the levels of vitamins and the astaxanthin concentration in the white muscle after slaughter (figure 5.3.10 and figure 5.3.14). There were also no correlations with the astaxanthin loss over the storage period (figure 5.3.11 and figure 5.3.15). This showed that the vitamins had no effect on the level of astaxanthin deposited in the flesh or on the loss of the pigment from the flesh during post-slaughter storage on ice.

The lack of significant results from this part of the trial was disappointing, leaving some questions unanswered. Without the analyses of the vitamin levels at the end of the storage period, it is not known whether astaxanthin plays a role in the protection of the two vitamins. Astaxanthin is a stronger anti-oxidant than ascorbic acid and α-tocopherol (Miki, 1991). It may therefore be supposed that it might play a part in sparing these anti-oxidants at its own expense. If this were so, it would be very hard for some astaxanthin not to be lost from the flesh during storage assuming that there is oxidation of the lipids within the flesh. Oxidation of the lipids within the flesh occurs, even during storage at low temperature. The corresponding production of free radicals will use up the anti-oxidants in the flesh, with the most reactive forms being used more rapidly.

Several lessons can be learnt from this part of the trial. The trial too ambitious for the facilities available. The slow extraction procedure for the α-tocopherol and the length of the run on the column required to separate the ascorbic acid meant that the samples had to be stored for considerable lengths of time before being fully analysed. The original plan had been to carry out astaxanthin, ascorbic acid and α-tocopherol extractions on all thirty samples from each cage at three time points during the storage. This had to be reduced when the length of the processes was taken into account. However, there is a balance required between costs and statistical accuracy. Several previous experiments on the effects of α-tocopherol on flesh quality, notably Sigurgisladottir *et al.*, (1994) used very low numbers of animals per treatment group. Given the degree of natural variability observed in salmon, it is unsurprising that no significant effects on pigment levels were found in such experiments. If real effects are to be found enough animals must be used, either for a regression style analysis as was used in this case, or in terms of treatment groups.

The use of several diets to generate the spread of vitamin contents in the flesh was successful. However, in retrospect it would have been better to have investigated the

effect of the level of one vitamin at a time. This could have been done using two or three diets, rather than the five used in the current trial. Such a trial would have still resulted in a wide variation in vitamin content, which is required to allow a regression analysis to be carried out (Zolman, 1993).

5.4.3 Effect of the Anti-oxidants on Eating Quality

Very few of the eating quality attributes were correlated with the concentration of the anti-oxidants in the flesh after slaughter (table 5.3.8). Of those that were, while an effect may have been found before storage on ice, none was found after storage.

Only one texture attribute was significantly affected by the level of the anti-oxidants. *Chewy* texture increased significantly with increasing α-tocopherol ($p<0.05$), but the degree of variation in the texture attribute was less than 5%. It had been expected that ascorbic acid could affect texture as it is involved in biosynthesis of collagen, but no effects were found to be caused its concentration. This could be because collagen biosynthesis was not limited during growth by the availability of ascorbic acid, which is supported by the observations that there were no effects of the diets on the health of the fish over the experiment. Several flavours were affected by the anti-oxidants. *Creamy* was negatively correlated with the concentration of ascorbic acid ($p<0.01$), which corresponds with the vitamin's acid flavour, but only 9% of the variation was accounted for. Other flavours commonly associated with sharp tasting compounds, such as *bitter*, *sour* and *metallic*, were unaffected.

Metallic flavour decreased with increasing astaxanthin and α-tocopherol ($p<0.05$), but in each case less than 5% of the variation in the attribute was accounted for. Surprisingly there was no affect of the anti-oxidants on *stale* flavour. *Stale* flavours are generally associated with products of lipid oxidation (Quarmby and Ratkowsky, 1988), and it was thought that the anti-oxidants would reduce such oxidation.

Certainly in red meat species the oxidation of oxymyoglobin to metmyoglobin is reduced by increasing the supplement of anti-oxidants in the diet (Liu *et al.*, 1995; Wood and Enser, 1997) and in trout increased α-tocopherol has been shown to reduce the potential for lipid oxidation (Frigg *et al.*, 1990).

In conclusion, in this experiment the level of the anti-oxidants in the flesh after slaughter had no real effects on the eating quality of the fish, either before or after storage on ice for 12 days. This has to be taken in conjunction with the warning applied to the previous section concerning the accuracy of the vitamin analyses.

5.4.4 Effect of Storage on Eating Quality

During the storage of foods various enzymatic, bacterial and oxidative reactions occur. These can enhance the desired textures and flavours of some foods, but generally the aim of storage is to preserve the product as it was before storage commences (Quarmby and Ratkowsky, 1988).

During storage of fish on ice the raw flesh becomes less firm to the touch (el Marrakchi *et al.* 1990) and the texture of the cooked fish is also less firm as assessed by a taste panel (Johansson and Kiessling, 1991). This was confirmed by the current trial, which found that the flesh of stored fish disintegrates more on biting and on chewing (higher *disintegration* and *dissolubility* ratings). However, in this trial there was no change in *firm* texture on cutting and, furthermore, *firm* texture on biting increased slightly with storage ($p<0.05$). These appear to be contradictions and cannot be explained.

The texture attributes related to liquid mouth feels were also significantly altered with storage. Johansson and Kiessling (1991) found that the *juiciness* of the flesh decreased with both storage on ice and at -18°C. However, the current trial found that *moist* texture increased with storage ($p<0.05$). Also other apparently related textures,

slimy, sticky and *gelatinous* increased ($p<0.001$, $p<0.05$, $p<0.001$ respectively). This supports the idea that the liquid mouth feel of the flesh increases with storage.

The *chewy* and *fibrous* textures of the fish do not change with storage ($p>0.05$), but the cohesion of the flesh does. Chapman *et al.*, (1993) found that the cohesiveness of cooked mackerel (*Scomber scombrus*) and hake (*Urophycis tenuis*) increased with frozen storage at -30°C over 24 months. However, the current trial showed that the *cohesiveness* of the cooked samples decreased with storage ($p<0.05$). This could be due to the nature of the flesh of the different species.

Flavours are significantly affected during storage. This has long been known and some of the causes have been identified. Generally desirable tastes (related to the presence of IMP) decrease and off-volatile (related to oxidation) and off-tastes (related to the presence of Hx and bitter peptides) increase with storage (Quarmby and Ratkowsky, 1988).

The current trial showed that with storage *creaminess* and *earthy* flavours decrease ($p<0.001$ and $p<0.01$ respectively). This could be caused by a real decrease in their strength, or by the increase of other flavours which then mask them. There were significant increases in *fishy, bitter, oily, sour, metallic, stale* and *salty* flavours ($p<0.001$) after storage. *Bitter* and *sour* flavours are associated with Hx and the bitter peptides and *oily* and *stale* flavours with oxidation (Quarmby and Ratkowsky, 1988). The cause of the increase in *salty* flavour is not known. These results support the findings of Johansson and Kiessling (1991) who demonstrated a significant decrease in *fresh* flavour after one week of storage on ice ($p<0.001$). However, these authors also found no effect on *bitter* flavour and an increase in *muddy* flavour after two weeks of storage. The lack of effect on *bitter* flavour contrasts with the knowledge that it is caused by the presence of Hx which increases with time post-slaughter, being

a breakdown product of IMP. The authors offer no explanation for this, nor for the increase in *muddy* flavour.

The overall eating experience was summed up using the hedonic rating of *overall flavour* and *overall liking*. Both of these ratings decreased significantly with storage ($p<0.001$). This shows that there is a perceptible change in the eating quality of the fish with storage on ice and that it is not liked by the taste panellists.

5.4.5 Factors Affecting Individual Attributes

The use of elementary linkage analysis (ELA) allowed the interactions between the individual attributes to be assessed. The attributes fell into four clusters, the largest of which was the first (figure 5.3.16). This showed that the relationship between *overall flavour* and *overall liking* is very strong ($r=0.945$). It also showed that *overall flavour* in the fresh fish is strongly correlated with *fishy* flavour and negatively correlated with *stale* flavour ($p<0.001$). Both *fishy* and *stale* flavours increase with storage, but the *overall flavour* decreases. This suggests that the relationship changes with storage.

Creaminess is very important to *overall liking*, being affected by the level of lipid in the flesh and the *bitter* flavour of the flesh. The texture of the flesh is also significant to the *overall liking*. *Sticky* and *disintegration* are both important attributes, although *disintegration* is negatively correlated with *overall liking*, while *sticky* is positively correlated. With storage *creaminess* decreases and *bitter* increases. *Disintegration* also increases greatly with storage and the *overall liking* decreases, confirming these relationships after storage.

The second cluster shows that there are strong correlations between *firmness* and *chewiness* as would be expected. Also *cohesion* and *dissolubility* are negatively correlated, which again would be expected from the definitions of these attributes.

From the third cluster the strong correlation between *slimy* and *gelatinous* textures can be seen. Similarly in the fourth cluster *metallic* is negatively correlated with *sweet.* Both of these correlations would be expected. Other correlations, which are shown in the clusters but are not discussed, are very poor. Although they are statistically significant ($p<0.05$), this level of significance is caused by the large number of samples. By squaring the correlation coefficient r the degree of variation is shown to be less than 15%, which does not describe a real relationship. All of the correlations with the chemical composition parameters investigated fell into this category. This showed that none of the four parameters investigated had real effects on the eating quality of the fresh cooked fish.

5.4.6 Conclusions

In conclusion, ascorbic acid or α-tocopherol levels in the flesh had no effects on the level of astaxanthin in the flesh after slaughter or after six and twelve days of storage on ice. The levels of these three anti-oxidants had no effect on the eating quality of the cooked flesh, either after slaughter or after storage on ice for twelve days. However, there were major changes in the eating quality of the fish during storage, with the fish becoming softer in texture, lower in *overall flavour* and lower in *overall liking*.

Chapter 6

Overall Discussion

The aim of this thesis has been to investigate some factors which may effect the flesh quality of salmonids. The composition, pigmentation and eating quality of the flesh are areas of particular interest as they are commercially important.

The literature review of flesh quality in salmonids highlighted where research had already been carried out. Building on the findings of the previous research and on work carried out on the flesh quality of other food animals the plan for the experimental part of the thesis was drawn up.

It was felt that, in the absence of background information, the thesis should be extensive in its range. Several hypotheses were put forward and experiments designed to test these. The results from these experiments are discussed in chapters 2 to 5. This chapter aims to draw out the major conclusions from these chapters and outline their importance for the salmonid farming industries as well as for researchers in this field.

6.1 Dietary Oil

The improved growth rates and feed conversion ratios achieved in the recent history of fish farming is largely the result of improvements in the diet formulation and manufacture. These improvements have allowed the diets to be adjusted to meet the requirements of the fish for vitamins, protein and energy. Once these demands were met, the diets were further adjusted to allow faster growth of the fish and to attempt to improve their flesh quality.

In order to grow fish require energy and protein. The protein levels of the old diets were already high, but the energy input was limiting the rate of growth. Therefore

greater levels of oil were added to the diets to increase growth rate. However, there are currently concerns that this may affect the composition of the flesh.

In other food animals it is known that increasing dietary oil results in increased lipid in the flesh of the animal. However, early experiments seemed to indicate that this was not the case in fish. As these findings were contrary to the results found from other farmed animals, this thesis has attempted to re-investigate this.

In this study, it was found that increasing the level of dietary oil resulted in faster growing fish, as was already known. However, these fish had a greater deposition of lipid in their viscera which led to a reduced yield after evisceration. The economic implications of this were not calculated, as this was not part of the experiment, but it would be a fairly simple task to determine whether the gains resulting from the increased growth rate outweighed the losses at evisceration.

The increased dietary oil also significantly raised the lipid content of the fillets in this trial. This has potential implications for the quality of the flesh. Increased lipid content has been shown to mask the pigment in the flesh and result in decreased colouration. Increased lipid has also been implicated in a condition known in the industry as soft flesh. This is an all-encompassing condition covering spongy textured flesh and flesh which is susceptible to gaping — the break up of the muscle structure. Unsightly free oil surrounding the vacuum-packed smoked product is also thought to be a result of high lipid levels.

The above three problems have been cited by the processing industry as reasons for reducing the level of lipid in the flesh. However, other factors could compound these conditions and there are good reasons, as shown in section 6.2, for keeping the high flesh lipid levels.

This study has shown the effect of the dietary oil level on the proximate composition of the fish. However, with the change in oil level, there would have been differences in the total energy content of each diet. Future investigations could study the effects resulting from using iso-calorific diets with varying quantities of oil. This would remove the effect of energy and concentrate just on the oil level. However, for the purposes of the current trial, it was felt that this was not necessary, as there were questions about the effects of the new range of high oil diets, which have much greater oil and energy levels than the old diets.

Further research could also address the quality concerns with regards to high lipid levels in the fish. Fish fed different levels of oil in the diet could be examined for their textural properties and the colour of the flesh determined. The amount of free oil surrounding the fish could also be investigated. Such a study would build on the information gained on the effects of the high oil diets on the quality of the flesh from the work described here.

6.2 Effect of Flesh Lipid on Eating Quality

The effect of flesh lipid on the eating quality of other food animals has been described. For example, in pork the level of lipid in the flesh was reduced by selective breeding with the aim of producing a healthier, lower-fat product by decreasing the amount of back fat. The unfortunate result of this was to reduce the flavour and juiciness of the pork.

Previous work at Langford had shown that flesh lipid levels affected the eating quality of cooked rainbow trout. However, little was known about the effects of lipid content

on the eating quality of salmon. This knowledge is important so that the problems encountered in other food industries can be avoided.

In this study it was found that the effects of lipid levels on the eating quality of the smoked and cooked salmon products were very different. The eating quality of cooked salmon was not affected by the level of lipid in the flesh. Thus there would appear to be a difference between trout and salmon flesh and this would suggest that trout is of limited value as a model for salmon.

However, the eating quality of smoked salmon was affected by the lipid content of the flesh. Both the texture and flavour of the flesh were altered by increasing the lipid level. The flesh became perceptibly softer to cutting, biting and chewing. Interestingly, these properties were liked by the taste panellists. The *salty* flavour of the flesh decreased markedly with increasing lipid. This and the other changes in flavours with increasing lipid were liked by the panellists. Thus the overall impression gained from the trial was that lipid content is very important to the eating quality of the smoked product and that the panellists favoured the higher lipid flesh.

The next step to be taken in any follow-up work would be to extend the fatness range investigated. This would then cover the whole range of fatnesses encountered in the industry, and could use the higher fat cuts from the belly flaps. From this, the next step could be a consumer trial. This would determine whether the high lipid flesh is really liked by consumers. If it is, then the current increase in dietary oil levels discussed in section 6.1 is having a beneficial effect on the liking of smoked salmon by increasing flesh lipid levels. If the high lipid levels are preferred by the consumers, the industry also needs to deal with the problems which are currently associated with high lipid flesh — *i.e.* poorly coloured and soft flesh and the loss of oil from the vacuum-packed product. The flesh of the smoked product gets softer as the lipid level increases, which will lead to changes in the ability to slice the product. However, this and the other problems could also be affected by other factors, such as

stress and growth rate, which may be controllable. Alternatively, the industry may need to develop new technology to deal with the high lipid product if it aims to supply a product with good flavour.

6.3 Pre-slaughter Handling and Slaughter Techniques

Common concerns amongst fish buyers and processors about the flesh quality of salmon include comments on the colour and texture of the flesh. Despite feeding the fish with well pigmented diet the flesh can appear pale and be soft to the touch. This has often been blamed on the level of oil in the diet, as discussed above in section 6.2. However, the problems of poor colour are not consistent, which implies that the diets are not the sole cause of the problem.

In the pork industry a condition often arises which results in poor flesh quality. Known as PSE (pale, soft, exudative) the flesh is much lighter in colour and softer than 'normal' and there is also a greater loss of exudate from the flesh. The condition is caused by acute stress or physical activity pre-slaughter.

The slaughter of salmonids under current commercial conditions appears highly stressful, involving either their transfer to a bath of carbon dioxide saturated water or to air, both of which result in strong aversive reactions by the fish. The degree of stress is also increased by crowding the fish, often for long periods, before slaughter.

Experiments discussed in chapter 4 showed that increasing the stress to the fish immediately prior to slaughter compromised the flesh quality. The time to rigor dropped dramatically, resulting in the most stressed fish entering rigor almost immediately after slaughter. Under commercial conditions, these fish would have

been eviscerated while in full rigor. This leads to the conclusion that many fish are handled and eviscerated commercially while in full rigor, which is known to result in damage to the flesh.

Increased stress at slaughter also resulted in less red-coloured, and paler, flesh. Furthermore, the flesh is more susceptible to gaping with handling. This could be further compounded if the fish are handled during full rigor. Thus it was shown that stress at slaughter caused the loss of flesh quality measured by two different parameters.

These results are of importance to the industry as they show the relationship between welfare and flesh quality. The welfare of fish has not been an emotive subject so far and so it has tended to be neglected. However, these results have shown that compromising the welfare has a direct effect on the quality of the flesh and increases the number of fish which fail the flesh quality criteria demanded by processing companies and the major retailers. This gives the salmon industry a clear indication that the welfare of the fish, especially at slaughter, should be considered.

Current 'best practice' methods of slaughter do not control the problem and may not be strictly adhered to. Therefore alternative slaughter methods need to be investigated. Some research in this area is currently being funded in Norway, but the results are not expected to be published until 2001. Further work is required to evaluate the current methods of slaughter. This would measure the time taken to kill the fish and the degree of stress associated with each method. From this an improved method could be devised to minimise the stress further. If the method could be automated this could reduce the handling time further and also provide a reproducible slaughter, as manual methods vary with time, due to fatigue of the operators. It would also be important to evaluate the effects on flesh quality of the new methods.

6.4 Effect of Anti-oxidants During Storage

Having concentrated on the effects of dietary and pre-slaughter treatments on flesh quality, the final studies investigated some changes in flesh quality post-slaughter. After slaughter the flesh has to be stored for a period before it is eaten. During this period it is susceptible to oxidation, which results in the deterioration of the flesh and possibly the loss of pigment. The primary aim of these experiments was to see whether it was possible to reduce post-mortem loss of pigment by increasing the levels of anti-oxidants in the flesh.

The anti-oxidant vitamins C and E were added in different levels to the diet of Atlantic salmon for ten weeks prior to slaughter. The levels of the vitamins at slaughter were then determined and correlations with these and with the level of astaxanthin in the flesh sought. From these results it was clear that there were no effects of the level of vitamins in the flesh on the level of astaxanthin either before or after storage. However, it did appear that the level of astaxanthin in the flesh immediately after slaughter increased with increased vitamin C and E in the diet. This effect could not be confirmed due to the structure of the trial and requires further investigation.

It was interesting to observe that during 12 days of storage on ice there was no significant decrease in the level of astaxanthin in the flesh. This confirmed a previous experiment by other researchers, but apparently conflicted with the observations from the industry which have reported the loss of colour from the flesh during storage. However, flesh colour can be affected by many factors other than the level of pigment. Changes in muscle structure during storage may result from high levels of pre-slaughter stress, which could cause the loss of colour. This would relate to the work carried out in chapter four, as discussed in section 6.3.

In contrast to the anti-oxidants, there were changes in the eating quality of the flesh with storage. The flesh stored on ice had more bitter flavours and a softer and greasier texture. This was found to be less liked by the taste panel.

There was found to be no effect of the levels of the anti-oxidant vitamins in the flesh immediately after slaughter on the eating quality before or after storage. This implied that either the vitamins were not affecting the rate of oxidation, or that the changes in eating quality were independent of the rate of oxidation.

Bitter flavours are associated with compounds such as hypoxanthine which is a breakdown product of ATP. The rate of production of hypoxanthine is increased by high levels of stress in the fish immediately prior to slaughter. Thus increased stress at slaughter could also increase the rate of production of off-flavours in fish, giving the flesh a shorter shelf-life. The onset of such flavours would be unaffected by the anti-oxidants within the flesh, but again highlights the importance of minimising pre-slaughter stress.

The work carried out in chapter 5 also questions the design of the experiment. The experiment was too big and too complicated for the resources available. With hindsight, a series of smaller experiments may have produced more useful results. Such experiments would have included measurements of the lipid oxidation during storage to see if there actually was an oxidation problem during storage. If the level of oxidation was low there would have been little effect of the vitamins which could explain the results found. Further work in this area should investigate this and find the effects of the vitamins in storage conditions where oxidation is a problem — possibly unwrapped fillets stored on illuminated shelves, as may be seen on a retailer's fish counter.

6.5 Conclusions from the Work

The results of the work carried out in this thesis have shown the importance of flesh lipid levels on the eating quality of smoked fish. Very little data on the eating quality of smoked salmon has been published, but this work has shown that lipid levels have important effects. This is important not only to the industry to ensure that eating quality is kept high, but to future researchers for the design of their experiments.

Perhaps the most important findings of this work came from the studies on pre-slaughter stress. The magnitude of the effect of increasing stress on the colour and texture of the flesh was large enough to have important implications for the salmon industry. Further research is required to determine the cause of these effects and to find ways to control them.

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Appendix 1

Feeding Chart for Fish in Chapter 2

This feeding regime is based on the EWOS Ltd. diet Vextra Classic, a 20% oil and 42% diet for rainbow trout.

Mean Fish Weight (g)	Temperature (°C) Feeding Rates — Percentage Body Weight Per Day				
	8	10	12	14	16
60 - 100	1.62	1.83	1.97	2.11	2.25
100 - 200	0.92	1.20	1.41	1.55	1.69
200 - 300	0.62	0.82	0.98	1.16	1.20

Appendix 2

Feeding Chart for Fish in Chapter 3 and Chapter 5

This feeding regime is based on the BOCM Pauls diet Fulmar Hyper, a 28% oil and 42% protein diet for salmon. The diets were fed at a rate determined by the mean weight of the fish in the cages.

Mean Fish Weight (g)	Temperature (°C) Feeding Rates — Percentage Body Weight Per Day					
	8	10	12	14	16	18
1500 - 2500	0.8	0.9	1.0	1.1	1.1	1.0
> 2500	0.6	0.7	0.8	0.9	0.9	0.8

Appendix 3

Process for Elementary Linkage Analysis (ELA)

Taken from course notes for the Multivariate Module (Year 2) of the Applied Statistics MSc (1997-1998), Sheffield Halham University.

3.2 Clustering of Variables

It may well be that in a multivariate analysis it is considered desirable to reduce the number of variables analysed or it may be of interest to see which variables 'go together' in some way. The most obvious measure of proximity between variables is the product moment, correlation coefficient, and so the correlation matrix provides a matrix of proximities for variables. The usual way of grouping these variables is via the 'typal' methods devised by L.L. McQuitty (1957, 1961, 1966) and the following example is taken from Philip et al (1975 Ch.. 10). We only consider the quickest and easiest method: Elementary Linkage Analysis (ELA) on the following matrix of social indices for 30 electoral wards for a certain town.

Table 3.1 Correlations between social indices

		(1)	(2)	(3)	(4)	(5)	(6)	(7)
Adolescent Psychiatric Referrals	(1)	1.00	0.77	0.12	0.11	0.09	0.55	0.41
Adolescent Attempted Suicide	(2)	0.77	1.00	0.14	0.03	0.07	0.60	0.40
Juvenile Delinquency	(3)	0.12	0.14	1.00	0.65	0.59	0.10	0.17
Overcrowding	(4)	0.11	0.03	0.65	1.00	0.52	0.09	0.20
Local Authority Housing	(5)	0.09	0.07	0.59	0.52	1.00	0.12	0.21
Suicide	(6)	0.55	0.60	0.10	0.09	0.12	1.00	0.45
Road Accidents	(7)	0.41	0.40	0.17	0.20	0.21	0.45	1.00

The ELA for these artificial data consists of five steps:

1. Underline the highest (absolute) entry in each column of the matrix — ignoring unities in the main diagonal. (This has been done in the above matrix).

2. Select the highest entry of the entire matrix; the two variables having this correlation constitute the first two variables of the first cluster. (The correlation is 0.77 and the variables are (1) and (2)).

3. Select all those variables which are most like the variables in the first cluster. Do this by reading along the rows of the variables which emerged in step 2 and select any underlined coefficients in these rows. (Coefficient 0.60 is underlined for row (2) and so variable (6) is selected).

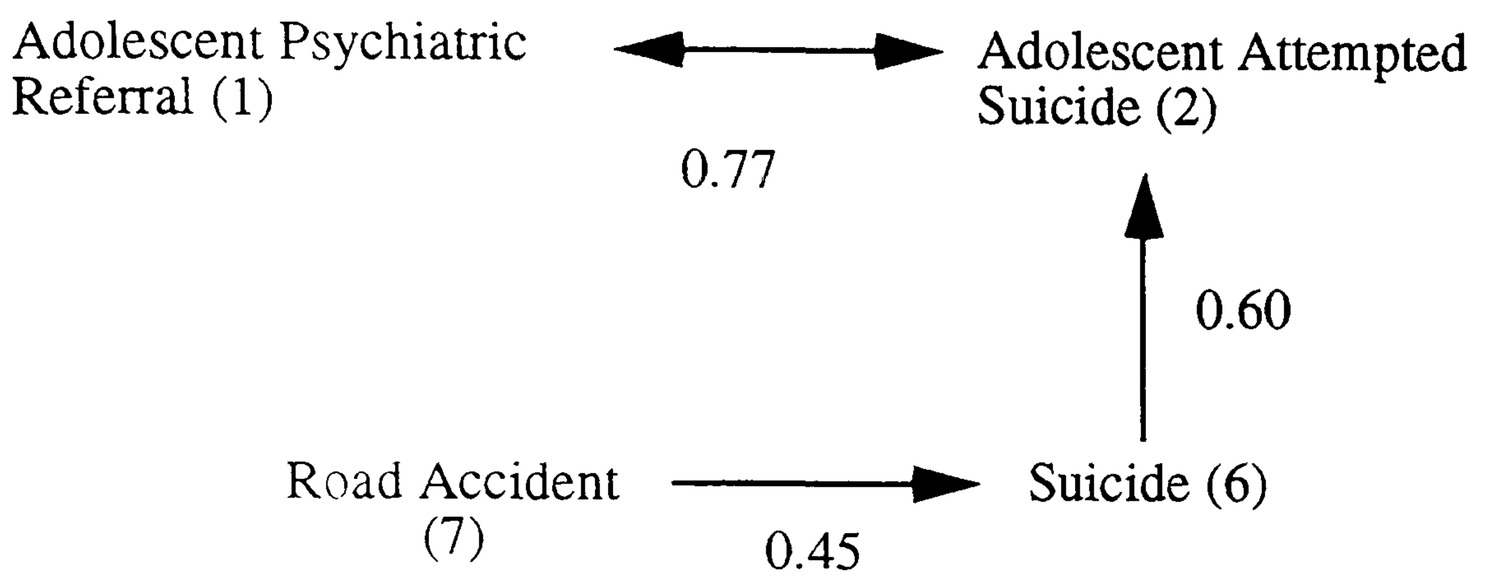
4. Select any variables which are most like those elicited at step 3. If any variables emerge, continue the selecting process until no further variables emerge. (Reading along row (6) shows coefficient 0.45 is underlined. Variable (7) is therefore selected and no further variables emerge).

5. Excluding all those variables which fall within the first cluster, repeat steps 2 to 4. (Variables (1), (2), (6) and (7) are excluded as these consist cluster one. The highest remaining underlined coefficient is 0.65 for variables (3), (4) and these form the initial variables for cluster two. Reading along row (3) shows 0.59 underlined and so variable (5) joins cluster two. No more variables can emerge and the analysis is complete).

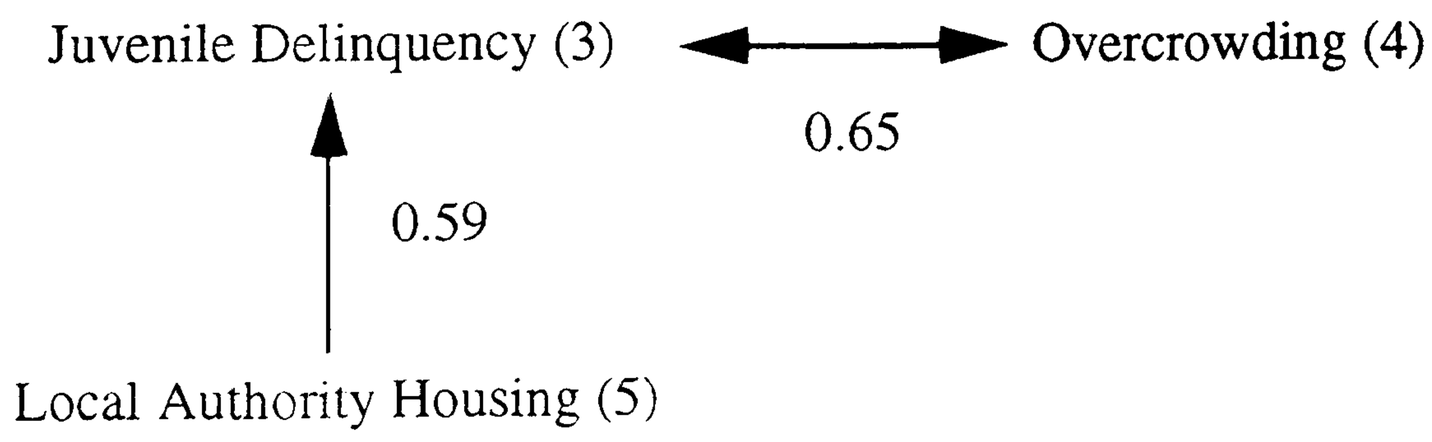
The results of the ELA can be shown as follows:

Figure 3.3

Cluster 1



Cluster 2



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